Clinical Trial Protocol

Protocol Title:	A Multi-Center, Randomized, Double Masked, Placebo Controlled Clinical Study to Assess the Safety and Efficacy of 0.1% RGN-259 Ophthalmic Solutions for the Treatment of Dry Eye Using the Controlled Adverse Environmental (CAE^{SM}) Model (ARISE-2)
Protocol Number:	RGN-259/16-110-0008 (NCT02974907)
Study Phase:	3
Product Name:	RGN-259 Ophthalmic Solution (0.1%)
IND Number:	73,446
Indication:	Dry Eye Syndrome (DES)
Investigators:	Multi-Center
Sponsor:	ReGenTree, LLC. 116 Village Boulevard, Suite 200 Princeton, NJ 08540 USA
Contract Research Organization:	Ora. Inc. 300 Brickstone Square, 3rd Floor Andover, MA 01810 USA
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Confidentiality Statement

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SYNOPSIS

Protocol Title:	A Multi-Center, Randomized, Double Masked, Placebo Controlled Clinical Study to Assess the Safety and Efficacy of 0.1% RGN-259 Ophthalmic Solutions for the Treatment of Dry Eye Using the Controlled Adverse Environmental (CAE^{SM}) Model (ARISE-2)
Protocol Number:	RGN-259/16-110-0008
Study Drug:	• 0.1% RGN-259 Ophthalmic Solution • Placebo Ophthalmic Solution
Study Phase:	3
Study Objective:	The objective of this study is to compare the safety and efficacy of 0.1% RGN-259 Ophthalmic Solutions to placebo for the treatment of the signs and symptoms of dry eye.
Overall Study Design	
Structure:	Multi-center, double-masked, randomized, placebo-controlled study
Duration:	An individual subject's participation is estimated to be approximately 6 weeks (42 days)
Controls:	Placebo Ophthalmic Solution (Vehicle)
Dosage/Dose Regimen:	Subjects eligible to be randomized will receive one of the following treatments to be administered bilaterally QID for 28 days (from Visit 2 to Visit 5). • 0.1% RGN-259 Ophthalmic Solution • Placebo Ophthalmic Solution (Vehicle) During a 14-day study run-in period (for the purpose of subject selection) prior to randomization, all subjects will receive Placebo Ophthalmic Solution (Vehicle) bilaterally QID
Summary of Visit Schedule:	5 visits over the course of approximately 6 weeks • Visit 1 Day -14 1 day, Screening

	• Visit 2 Day 1, Confirmation / Baseline • Visit 3 Day 8 1 day, 1-Week Follow-Up • Visit 4 Day 15 1 day, 2-Week Follow-Up • Visit 5 Day 29 2 day, 4-Week Follow-Up and Study Exit
Measures Taken to Reduce Bias:	This is a randomized treatment assignment, double masked study.
Study Population Characteristics	
Number of Subjects:	Approximately 800 subjects will be screened to enroll approximately 594 (297 per treatment arm) subjects
Condition/Disease:	Dry Eye Syndrome
Inclusion Criteria:	**Subjects must:** a. Be at least 18 years of age; b. Provide written informed consent; c. Have a subject reported history of dry eye for at least [redacted] prior to Visit 1; d. Have a history of use or desire to use eye drops for dry eye symptoms within [redacted] of Visit 1; e. Report a score of [redacted] in at least one symptom on the Ora Calibra™ Ocular Discomfort & 4-Symptom Questionnaire assessed pre-CAE^{SM}, at Visits 1 and 2; f. Have a Schirmer's Test score of [redacted] at Visits 1 and 2; g. Have a Tear Film Break-Up Time (TFBUT)© [redacted] at Visits 1 and 2, pre-CAE^{SM};

h. Have a corneal fluorescein staining score of [redacted] according to the Ora Calibra™ Scale at Visits 1 and 2, pre-CAE[SM];

i. Have a total corneal fluorescein staining [redacted] according to the Ora Calibra™ Scale at Visits 1 and 2, pre-CAE[SM];

j. Have a total lissamine green conjunctival score [redacted] based on the sum of the temporal and nasal regions of the conjunctiva, according to the Ora Calibra™ Scale at Visits 1 and 2, pre-CAE[SM];

k. Demonstrate a response to the CAE[SM] at Visits 1 and 2 as defined by:

1. [redacted]

2. [redacted]

	l. Have at least one eye (the same eye) satisfy all criteria for f, g, h, i, j and k. above.
Exclusion Criteria:	**Subjects must not:** a. Have any clinically significant slit-lamp findings at Visit 1 that may include active blepharitis, meibomian gland dysfunction (MGD), lid margin inflammation or active ocular allergies that require therapeutic treatment, and/or in the opinion of the investigator may interfere with study parameters; b. Be diagnosed with an ongoing ocular infection (bacterial, viral, or fungal), or active ocular inflammation at Visit 1; c. Have worn contact lenses within 7 days of Visit 1 or anticipate using contact lenses during the study; d. Have used any eye drops within 2 hours of Visit 1; e. Have previously had laser-assisted *in situ* keratomileusis (LASIK) surgery within the last 6 months; f. Have used Restasis® or Xiidra™ within 45 days of Visit 1; g. Have an IOP > 25 mmHg at Visit 1; h. Have any planned ocular and/or lid surgeries over the study period; i. Be using or anticipate using temporary punctal plugs during the study that have not been stable within 30 days of Visit 1; j. Be currently taking any topical ophthalmic prescription (including medications for

	glaucoma) or over-the-counter (OTC) solutions, artificial tears, gels or scrubs, and cannot discontinue these medications for the duration of the trial (excluding medications allowed for the conduct of the study); k. Have corrected visual acuity greater than or equal to logMAR +0.7 as assessed by Early Treatment of Diabetic Retinopathy Study (ETDRS) scale in both eyes at Visit 1; l. Have an uncontrolled systemic disease; m. Be a woman who is pregnant, nursing or planning a pregnancy; n. Be unwilling to submit a urine pregnancy test at Visit 1 and Visit 5 (or early termination visit) if of childbearing potential. Non-childbearing potential is defined as a woman who is permanently sterilized (e.g. has had a hysterectomy or bilateral tubal ligation or bilateral oophorectomy), or is post-menopausal (without menses for 12 consecutive months); o. Be a woman of childbearing potential who is not using an acceptable means of birth control; acceptable methods of contraception include: hormonal oral, implantable, injectable, or transdermal contraceptives; mechanical spermicide in conjunction with a barrier such as a diaphragm or condom; IUD; or surgical sterilization of partner. For non-sexually active females, abstinence may be regarded as an adequate method of birth control; however, if the subject becomes sexually active during the study, she must agree to use adequate birth control as defined above for the remainder of the study;

	p. Have a known allergy and/or sensitivity to the study drug or its components; q. Have a condition or be in a situation which the investigator feels may put the subject at significant risk, may confound the study results, or may interfere significantly with the subject's participation in the study; r. Be currently enrolled in an investigational drug or device study or have used an investigational drug or device within 30 days of Visit 1; s. Be currently using any medication known to cause ocular drying (e.g. antihistamines, anti-depressants) or increased lacrimation (e.g. cholinergics) that is not used on a stable dosing regimen for at least 30 days prior to Visit 1; t. Be receiving systemic corticosteroid therapy (not including inhaled corticosteroids), ophthalmic topical steroids, immunotherapy, or cytotoxic therapy within 14 days of Visit 1 or anticipate such therapy throughout the study period. u. Be unable or unwilling to follow instructions, including participation in all study assessments and visits.
Study Formulations:	• 0.1% RGN-259 Ophthalmic Solutions • Placebo Ophthalmic Solution
Efficacy Measures:	**<u>Primary Efficacy Measures:</u>** • Change from baseline in ocular discomfort change from pre-CAE℠ [redacted] to post CAE℠ [redacted] at Day 29 (Visit 5) using the Ora Calibra™ Ocular Discomfort Scale; <u>Hierarchical Efficacy Measure:</u>

	• Change from baseline in [redacted] staining at Day 29 pre-CAE[SM] in [redacted] using the Ora Calibra™ scale. **<u>Secondary Efficacy Measures:</u>** • Fluorescein staining (Ora Calibra™ scale) at Visits 3, 4, and 5 (Change from Pre-CAE[SM] to Post-CAE[SM], Pre- and Post-CAE[SM]; regions: [redacted]; • Fluorescein staining (Ora Calibra™ scale) at Visits 3, 4, and 5 (Change from Pre-CAE[SM] to Post-CAE[SM], Pre- and Post-CAE[SM]; regions: [redacted].; • Lissamine green staining (Ora Calibra™ scale) at Visits 3, 4, and 5 (Pre- and Post-CAE[SM]; regions: [redacted] • Tear film break-up time at Visits 3, 4, and 5 (Pre- and Post-CAE[SM]); • Ocular Protection Index (OPI 2.0) at Visit 5 (Pre-CAE[SM]); • Unanesthetized Schirmer's Test at Visit 5 (Pre-CAE[SM]); • Drop comfort assessment after randomization at Visit 2;

	• Ocular Surface Disease Index (OSDI)© at Visits 3, 4, and 5 (Pre-CAE^{SM}); • Ocular discomfort and dry eye symptoms at Visits 3, 4, and 5 (Change from Pre-CAE^{SM} to Post-CAE^{SM}, Pre- and Post-CAE^{SM}); • Ocular discomfort during CAE^{SM} at Visits 4 and 5; • Daily diary.
Safety Measures:	• Visual acuity (ETDRS) at Visits 1, 2, 3, 4 and 5; • Slit-lamp biomicroscopy at Visits 1, 2, 3, 4 and 5; • Corneal Sensitivity at Visits 1 and 5 (Pre-CAE$^{SM)}$); • Adverse event query at Visits 1, 2, 3, 4 and 5; • Undilated Fundoscopy at Visits 1 and 5; and • Intraocular Pressure at Visits 1 and 5.
Other:	Not applicable.
General Statistical Methods and Types of Analyses Analysis Populations • Intent-to-Treat Population The intent-to-treat (ITT) population includes all randomized subjects. The primary analysis will be performed on the ITT population with the Last Observation Carried Forward (LOCF) imputation method for missing values. The ITT population may also be analyzed with observed data only (i.e., without LOCF) to assess sensitivity. Subjects in the ITT population will be analyzed as randomized. • Per Protocol Population The per protocol (PP) population includes subjects in the ITT 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 efficacy variables. Subjects in the PP population will be analyzed as randomized.

- Safety Population The safety population includes all 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.

Randomization

At Visit 2 (Day 1), subjects will be randomized in a 1:1 ratio to receive either 0.1% RGN-259 or placebo. Randomization will be stratified by the following factors:

1. Change Pre-CAE[SM] [redacted] to Post-CAE[SM] [redacted] in ocular discomfort (Ora Calibra Ocular™ Discomfort Scale) at Visit 2.

[redacted]

2. Pre-CAE[SM] [redacted] fluorescein staining (Ora Calibra ™ Scale) at Visit 2

[redacted]

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 worst eye will be the eye with the largest increase in ocular discomfort from pre-CAE[SM] [redacted] to post-CAE[SM] [redacted] at Visit 2. If the ocular discomfort symptom increase is the same in both eyes then the worst eye will be the eye with worse (higher) [redacted] staining pre-CAE[SM] at Visit 2. If the [redacted] staining is the same in both eyes then the right eye will be selected as the worst eye.

Sample Size and Hierarchical Analysis Approach

This study is expected to enroll 297 subjects in each of the two treatment arms, for a total of 594 randomized subjects. Assuming a 5% drop out rate, 282 subjects per group are expected to complete the study.

The primary endpoint is the change from baseline in ocular discomfort change from pre-CAE[SM] [redacted] to post-CAE[SM] [redacted] at Day 29. The study will be considered successful if and only if this endpoint is met. With [redacted] subjects randomized per treatment arm, and a difference between RGN-259 and placebo as low as [redacted], the study will have [redacted] power to detect a treatment difference. This assumes a two-sided test at alpha 0.05 and a common standard deviation (SD) of [redacted] in both treatment arms. Accounting for up to 5% dropouts in the study, 282 subjects randomized per treatment arm, or 564 total subjects, will have [redacted] power to detect a difference for the primary endpoint. Difference and SD estimates are derived from the phase 2b/3 study.

The hierarchical endpoint of the change from baseline in [redacted] staining at Day 29 pre-CAE[SM] is also considered in terms of statistical power. It is assumed that 50% (282 completed subjects accounting for 5% dropouts) of the subjects will have a baseline [redacted]. This endpoint will be tested conditionally upon the primary endpoint being successful, thereby maintaining the study-wide type I error rate for both endpoints. With a sample size of [redacted] subjects per treatment arm with a [redacted] (50% of the randomized subjects) the study will have [redacted] power to detect a difference as low as [redacted] between RGN-259 and placebo. This assumes a two-sided test at alpha 0.05 and a common SD of [redacted] in both treatment arms. To show a range of power using the same assumptions and accounting for fewer subjects with a [redacted] if only 35% of subjects (99 completed subjects per treatment arm) have a baseline [redacted] then the study will have 83% power to detect a treatment difference between RGN-259 and placebo. Difference and SD estimates are derived from the phase 2b/3 study, in a [redacted]

Primary Efficacy Analyses:

Change from baseline in ocular discomfort change from pre- to post-CAE[SM] at Day 29 (Visit 5) using the Ora Calibra™ Ocular Discomfort Scale will be calculated as:

(OD[redacted] at Day 29 OD[redacted] at Day 29) (OD[redacted] at Baseline OD[redacted] at Baseline)

Where OD[redacted] Ocular discomfort at [redacted] in CAE (pre-CAE) and OD[redacted] Ocular discomfort at [redacted] in CAE (post-CAE)

With this calculation, a positive change indicates a worsening of dry eye symptoms compared to baseline. In addition, treatment comparisons between active and placebo will be calculated as active placebo, such that a negative result indicates a

better score for the active treatment (i.e., the active treatment had a smaller increase in dry eye symptoms during the CAE[SM] compared to baseline than the placebo group).

ANCOVA models will be used to compare the change from baseline to Day 29 (Visit 5) in the change from pre-CAE[SM] to post-CAE[SM] in ocular discomfort, as measured on the Ora Calibra™ Ocular Discomfort Scale, between 0.1% RGN-259 Ophthalmic Solution and Placebo. The ANCOVA models will include terms for baseline change from pre-CAE[SM] to post-CAE[SM] ocular discomfort and study site. In addition, the study site by treatment interaction will be explored in a separate model to evaluate how the treatment effect may differ across study sites. The primary analysis will use LOCF imputation to have a full accounting of the ITT population at the Day 29 visit.

Hierarchical Efficacy Analysis

Conditional upon the discomfort endpoint showing statistical significance in favor of 0.1% RGN-259, the [redacted] staining endpoint will be tested. ANCOVA models will be used to compare the change from baseline to Day 29 (Visit 5) in [redacted] staining using the Ora Calibra™ scale (pre-CAE) [redacted], between 0.1% RGN-259 Ophthalmic Solution and Placebo. The ANCOVA models will include terms for baseline pre-CAE [redacted] staining and study site. In addition, the study site by treatment interaction will be explored in a separate model to evaluate how the treatment effect may differ across study sites. The analysis will use LOCF imputation to have a full accounting of the ITT population at the Day 29 visit.

Various sensitivity analyses of the primary endpoints will be conducted to evaluate the impact of missing data, including analyses of observed data, imputing missing data using multiple imputation methods, and using the PP population.

Secondary Efficacy Analyses:

The continuous and ordinal secondary efficacy variables collected at each visit will be summarized descriptively (n, mean, standard deviation, median, min and max), and analyzed with two-sample t-tests comparing each of the active treatment groups to placebo. All visit-based data will be analyzed at each visit and change from baseline. A Wilcoxon rank sum test and an ANCOVA model adjusting for baseline and site will also be assessed where appropriate. No imputation will be performed for secondary efficacy variables.

Corneal fluorescein staining by region and total, lissamine green staining by region, TFBUT, ocular protection index, un-anesthetized Schirmer's test, Drop comfort

assessment, OSDI©, ocular discomfort and dry eye symptoms, ocular discomfort during CAE℠, and changes from baseline in these measures will be analyzed by visit using two-sample t-tests and Wilcoxon rank sum tests, as appropriate.

The worst symptom for each subject will be identified as the symptom with the highest daily average score during the run-in period (Days -14 to -1) as recorded in the subject diary. The worst symptom and each individual symptom will be analyzed using a two-sample t-test. This analysis will be completed separately for the morning and evening scores as well as the daily average score. Additionally, the average score for each time point (morning, evening, before bed and daily average) will also be analyzed separately using a Wilcoxon rank sum test.

Safety Variables

Adverse events will be coded using the MedDRA dictionary. Frequencies and percentages of subjects with treatment-emergent adverse events (TEAEs), serious TEAEs, and TEAEs causing premature discontinuation will be provided by treatment group. An AE is treatment emergent if it occurs or worsens after the first dose of study treatment. Furthermore, frequencies will be given of subjects with TEAEs by system organ class, by system organ class and preferred term, by system organ class, preferred term and maximal severity, by system organ class, preferred term and strongest relationship, and by system organ class, preferred term, maximal severity, and strongest relationship. Separate analyses will be performed for ocular specific and all AEs (including systemic).

Other safety endpoints including visual acuity, slit-lamp biomicroscopy, corneal sensitivity, undilated fundoscopy, and intraocular pressure will be summarized by treatment group and visit using descriptive statistics. Changes or shifts from baseline will also be summarized where appropriate. For assessments performed by eye, study eye and fellow eye will be summarized separately. In addition, shifts from baseline to worst on-treatment value for ocular safety assessments will be summarized.

Summary of Known and Potential Risks and Benefits to Human Subjects

There are no known risks with the instillation of RGN-259 (Tβ4 Ophthalmic solution). In a 28-day repeat dose ocular toxicity study in the rabbit, RGN-259 (Tβ4 Ophthalmic solution) at doses up to 0.1% produced no local or systemic toxicity and did not appear to induce ocular disease. A Phase 1 clinical trial evaluating the safety and tolerability of RGN-259 was not required.

Risks are minimal based on the safety profile of Tβ4 in nonclinical toxicology and safety pharmacology studies where systemic doses (≥ [redacted] mg/kg bw/day) produced no observed adverse events. In a Phase 1 study in healthy volunteers, systemic doses of Tβ4 injectable solution up to approximately [redacted] mg/kg bw/day yielded no dose limiting toxicities and no serious adverse events. Three unexpected and possibly related events dizziness (1 subject), headache (1 subject) and pyrexia (1 subject) were reported.

A total of 425 subjects have been enrolled in 5 clinical studies employing the 0.05% and 0.1% concentration of Tβ4 ophthalmic drops, and the drug has been shown to be safe and well-tolerated. The largest of these, a 317-subject trial of 0.05% and 0.1% Tβ4 ophthalmic drops for treatment of dry eye, reported no significant adverse effects and no clinically significant changes in any safety parameters.

A Phase 2b/3 trial proved RGN-259's ability to protect from adverse stimuli as indicated by the dampening of response from a baseline to 28-day CAE^{SM} challenge. RGN-259 caused significant lowering of scores in ocular discomfort and [redacted] corneal staining compared to placebo treatment.

This data indicates that RGN-259 ophthalmic solution represents a promising treatment for patients with dry eye disease.

Schedule of Visits and Measurements

Procedure	Visit 1 Day -14 ± 1		Visit 2 Day 1		Visit 3 Day8 ± 1	Visit 4 Day15 ± 1		Visit 5 Day 29 ±2	
	Pre CAE[SM]	Post CAE[SM]	Pre CAE[SM]	Post CAE[SM]		Pre CAE[SM]	Post CAE[SM]	Pre CAE[SM]	Post CAE[SM]
Informed Consent / HIPAA	X								
Medical / Medication History and Demographic	X								
Run in Placebo Collection			X						
Study Drug Collection					X	X		X	
Diary Collection			X		X	X		X	
Medical / Medication History Update			X		X	X		X	
Adverse Event Query		X	X	X	X	X	X	X	X
Pregnancy Test	X[1]							X[1]	
Ocular Discomfort Ora, Calibra™ / Dry Eye Symptoms	X	X	X	X	X	X	X	X	X
OSDI© Questionnaire	X		X		X	X		X	
Visual Acuity (ETDRS)	X		X		X	X		X	
Review of Qualification Criteria	X	X	X	X					
Slit lamp Biomicroscopy	X	X	X	X	X	X	X	X	X
Ocular Protection Index (OPI 2.0) Ora Calibra™ Scale			X					X	
TFBUT©	X	X	X	X	X	X	X	X	X
Fluorescein Staining Ora Calibra™ Scale	X	X	X	X	X	X	X	X	X

Lissamine Green Staining Ora Calibra™ Scale	X	X	X	X	X	X	X	X	X
Corneal Sensitivity (Cochet Bonnet)	X							X	
Unanesthetized Schirmer's Test	X		X		X	X		X	
CAE^{SM} Exposure	X		X			X		X	
CAE^{SM} Discomfort Ora Calibra™ Ocular Disc. Scale	X^2		X^2			X^2		X^2	
Tear Collection				X^3			X^3		X^3
Intraocular Pressure		X							X
Undilated Fundus Exam		X							X
Run in Placebo Dispensation		X							
Randomization				X					
Subject Self instillation of study drug				X	X		X		
Ora Calibra™ Drop Comfort Assessment				X	X		X		
Study Drug Dispensation				X	X^4		X		
Diary Dispensation		X		X	X		X		
Exit Subject from Study									X

X^1 For females of childbearing potential, **X**^{2} [redacted] **X**^{3} Tears may be collected after a 30 minute wait period from lissamine instillation, X^4 IP will be collected and re dispensed at visit 3.

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LIST OF ABBREVIATIONS

AE	adverse event
ANCOVA	analysis of covariance
BCVA	best-corrected visual acuity
CAE^{SM}	controlled adverse environment
CFR	Code of Federal Regulations
CI	confidence interval
CRF	case report form
DES	Dry Eye Syndrome
DHHS	Department of Health and Human Services
eCRF	electronic case report form
EKG	Electrocardiograph
ERC	ethical review committee
ETDRS	Early Treatment of Diabetic Retinopathy Study
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
HIPAA	Health Information Portability and Accountability Act
ICF	informed consent form
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
IND	investigational new drug application
IOP	intraocular pressure
IRB	institutional/independent review board
ITT	intent-to-treat
IUD	intra uterine device
IWRS	interactive web response system
KCS	keratoconjunctivitis sicca
Kg	Kilogram
LASIK	laser *in situ* keratomileusis
LOCF	last observation carried forward
logMAR	logarithm of the minimum angle of resolution
MedDRA	Medical Dictionary for Regulatory Activities
MGD	meibomian gland dysfunction
Mg	Milligram
mL	Milliliter
Mm	Millimeter
µg	microgram
µL	microliter
µm	Micrometer
mmHg	millimeters of mercury
OD	right eye
OPI	ocular protection index

OS	left eye
OSDI©	ocular surface disease index
OU	both eyes
OTC	over-the-counter
PMNs	polymorphonuclear leucocytes
PP	per protocol
QID	four times a day
SAE	serious adverse event
Ac-SDKP	N-acetyl-seryl-aspartyl-lysyl-proline
Tβ4	Thymosin Beta 4
TFBUT©/TBUT	tear film break-up time
TEAEs	treatment emergent adverse events
VA	visual acuity

1 INTRODUCTION

Dry eye is a complex disease that results in symptoms of discomfort, visual disturbance, and tear film instability which creates potential for damage for the ocular surface. It is accompanied by increased osmolarity of the tear film and inflammation of the ocular surface. Estimates of the prevalence of dry eye vary considerably, depending on the criteria used to define the syndrome. In the U.S., it has been estimated that as many as 3.2 million women and 1.7 million men over the age of 50 have dry eye, with a projected 40% increase in number of patients affected by 2030 (Schaumberg, Sullivan et al. 2002, Schaumberg, Sullivan et al. 2003, Smith 2007, Schaumberg, Dana et al. 2009). With the aging population in the United States and other countries of the developed world, and with increasing computer use, dry eye is expected to become more prevalent and finding a treatment is becoming more important (Brewitt and Sistani 2001).

Thymosin Beta 4 (Tβ4) is a synthetic copy of the naturally-occurring 43-amino acid peptide that is found in a variety of tissues and nucleated cell types. It is found in high concentrations in blood platelets (anucleated fragments of precursor megakaryocytes), macrophages, polymorphonuclear leucocytes (PMNs), and other lymphoid cells. PMNs and platelets are among the first formed elements to enter wounds. The mammalian gene encoding the expression of Tβ4 localizes to the X-chromosome, and it is the first gene to be upregulated during wounding.

Tβ4 is a major actin sequestering peptide and a potent regulator of actin polymerization in all eukaryotic cells. Actin has many important functions that, among other things, are essential for cell motility, microtubule organization, and construction of the cytoskeleton. These functions are regulated by Tβ4, which maintains a large pool of actin monomers, thus controlling the assembly and disassembly of actin filaments that regulate the dynamics of the actin cytoskeleton.

Tβ4 is released by platelets and is present in wound fluid (Huff et al., 2002) where it promotes wound repair and regeneration in various tissues. In the eye, it promotes corneal epithelial cell migration, decreases inflammation and has anti-apoptotic activities (Sosne, Siddiqi et al., 2004; Sosne et al., 2005; Sosne et al., 2007; Qui et al., 2007), and accelerates repair/regeneration after alkali burn and heptanol debridement (Sosne, Szliter et al., 2002). In the eye it up-regulates the gene expression of laminin-5, a major subepithelial adhesion protein, located in the basement membrane region of the cornea, conjunctiva, and skin, and important in wound healing (Sosne, Lihua et al., 2004). In compassionate use cases, Tβ4 has demonstrated efficacy in repairing non-healing neurotrophic corneal ulcers and other corneal epithelial wounds (Dunn et al., 2010, Sosne et al., 2010).

Ac-SDKP can reverse hypertension-induced fibrosis (Peng, 2003) and has anti-inflammatory activity, which is one possible explanation of how Tβ4 reduces fibrosis (Cavasin, 2006).

It is known that Tβ4 plays a role in vascular biology, acts as a chemo-attractant for endothelial cells and stimulates angiogenesis, promotes cell migration and has been shown to have anti-apoptotic properties.

Because of its high concentration and ubiquitous distribution, Tβ4 apparently function as an important intracellular structural element and has additional "moonlighting" functions, when released from cells either by secretion, cell death, or lysis (Goldstein et al., 2005).

Topically applied Tβ4 has been shown to be safe and well tolerated in phase 1 and phase 2 clinical trials in the skin (RGN-137, Tβ4 dermal gel) and the eye (RGN-259, Tβ4 preservative-free eye drops). Tβ4 injectable solution (RGN-352) is deemed safe and well tolerated in a phase 1 clinical trial (Ruff et al., 2010).

While there were no Phase 1 studies of ophthalmic formulations of Tβ4, a double-blind, randomized, placebo-controlled, dose response study was conducted using four cohorts with 10 healthy subjects each given a single intravenous dose of Tβ4 or placebo. Cohorts received ascending doses of [redacted] mg of Tβ4 (Phase 1A). Following safety review of the Phase 1A data, subjects in Phase 1B received the same doses administered to those in Phase 1A but daily for [redacted] consecutive days. Safety evaluations, incidence of unexpected adverse events (AEs) and PK parameters were evaluated. AEs were infrequent and mild and moderate in intensity. There were no deaths, no dose limiting toxicities or SAEs. The PK profile showed a dose proportional response, increasing half-life with increasing dose and minimal drug accumulation.

A Phase 2 dry eye clinical trial (RGN-DE-202) of 0.1% RGN-259 ophthalmic solution compared to placebo showed a decrease in ocular discomfort and corneal staining in subjects with dry eye as well as a decrease change in corneal staining following exposure to a controlled adverse environment which suggests that Tβ4 has a protective effect on the eye when used in an adverse environment.

A more recent Phase 2/3 study was completed and, while the primary endpoints were not met, several important measures of efficacy show that RGN-259 has a therapeutic effect in the treatment of dry eye. 0.05% RGN-259 and 0.1% RGN-259 ophthalmic solutions were shown to be well tolerated, as evidenced by the lack of significant differences in drop comfort assessment scores, and safe. Although the primary outcome measures were not met, several pre-specified endpoints and subgroups of subjects with more severe dry eye symptoms at baseline showed significant treatment effects, notably for the following endpoints: ocular discomfort during CAE[SM] [redacted] at Visit 5, improved fluorescein staining in the [redacted] at Visits 4 and 5, ocular discomfort at Visit 5, pre-CAE, and corneal fluorescein staining at Visit 5, pre-CAE[SM]. These results, especially those that were repeated from the previous Phase 2 study, suggest that RGN-259 has a therapeutic effect in the treatment of dry eye.

2 STUDY OBJECTIVES

The objective of this study is to compare the safety and efficacy of 0.1% RGN-259 Ophthalmic Solutions to placebo for the treatment of the signs and symptoms of dry eye.

3 CLINICAL HYPOTHESES

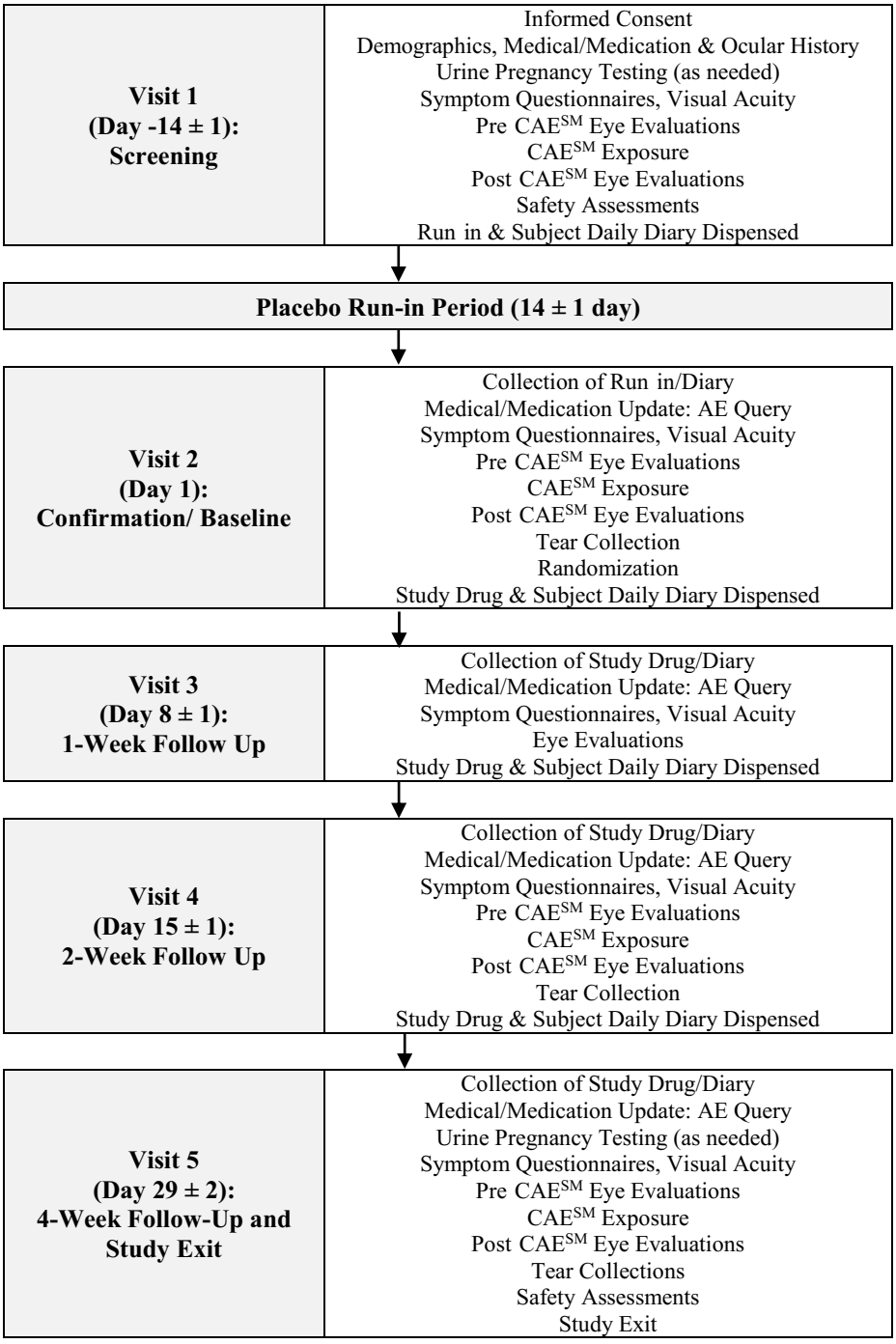
The clinical hypothesis for this study is that 0.1% RGN-259 ophthalmic solution is superior to placebo for the primary and hierarchical endpoints of signs and symptoms, as follows:

- Change from baseline in ocular discomfort change from pre-CAE[SM] [redacted] to post-CAE[SM] [redacted] at Day 29 (Visit 5) using the Ora Calibra™ Ocular Discomfort Scale;

- Change from baseline in [redacted] staining at Day 29 pre-CAE[SM] [redacted] using the Ora Calibra™ scale.

4 OVERALL STUDY DESIGN

This is a Phase 3, multicenter, randomized, double-masked study designed to evaluate the efficacy and safety 0.1% RGN-259 ophthalmic solution compared to placebo in subjects with dry eye. Approximately 594 male and female subjects at least 18 years of age with a subject-reported history of dry eye in both eyes and meeting all other study eligibility criteria will be randomized to receive treatment with RGN-259 or placebo in a 1:1 ratio (approximately 297 subjects in each treatment group). This study will consist of two periods: a 14-day run-in period and a 4-week treatment period. A study flow chart appears below:



Subjects who terminate early during the treatment period will be asked to complete safety assessments prior to commencement of any alternative dry eye therapy (if at all possible). Subjects who are terminated early from the study will not be replaced.

5 STUDY POPULATION

5.1 Number of Subjects (approximate)

It is estimated that approximately 800 subjects will be screened to enroll approximately 594 randomized subjects (297 in each arm). Subjects will be randomized in a 1:1 ratio 0.1% RGN-259 to placebo ophthalmic solution.

5.2 Study population characteristics

All subjects must be at least 18 years of age, of either gender, and of any race, and must meet all inclusion criteria and none of the exclusion criteria.

5.3 Inclusion Criteria

Subjects must:

a. Be at least 18 years of age;

b. Provide written informed consent;

c. Have a subject reported history of dry eye for at least [redacted] prior to Visit 1;

d. Have a history of use or desire to use eye drops for dry eye symptoms within [redacted] of Visit 1;

e. Report a score of [redacted] in at least one symptom on the Ora Calibra™ Ocular Discomfort & 4-Symptom Questionnaire assessed pre-CAE^{SM}, at Visits 1 and 2;

f. Have a Schirmer's Test score of [redacted] at Visits 1 and 2;

g. Have a Tear Film Break-Up Time (TFBUT)© [redacted] at Visits 1 and 2, pre-CAE^{SM};

h. Have a corneal fluorescein staining score of [redacted] according to the Ora Calibra™ Scale at Visits 1 and 2, pre-CAE^{SM};

i. Have a total corneal fluorescein staining of [redacted] according to the Ora Calibra™ Scale at Visits 1 and 2, pre-CAE^{SM};

j. Have a total lissamine green conjunctival score of [redacted], according to the Ora Calibra™ Scale at Visits 1 and 2, pre-CAE^{SM};

k. Demonstrate a response to the CAE^{SM} at Visits 1 and 2 as defined by:

   a. [redacted]

b. [redacted]

l. Have at least one eye (the same eye) satisfy all criteria for f, g, h, i, j and k. above.

5.4 Exclusion Criteria

Subjects must not:

a. Have any clinically significant slit-lamp findings at Visit 1 that may include active blepharitis, meibomian gland dysfunction (MGD), lid margin inflammation or active ocular allergies that require therapeutic treatment, and/or in the opinion of the investigator may interfere with study parameters;

b. Be diagnosed with an ongoing ocular infection (bacterial, viral, or fungal), or active ocular inflammation at Visit 1;

c. Have worn contact lenses within 7 days of Visit 1 or anticipate using contact lenses during the study;

d. Have used any eye drops within 2 hours of Visit 1;

e. Have previously had laser-assisted in situ keratomileusis (LASIK) surgery within the last 6 months;

f. Have used Restasis® or Xiidra™ within 45 days of Visit 1;

g. Have an IOP > 25 mmHg at Visit 1;

h. Have any planned ocular and/or lid surgeries over the study period;

i. Be using or anticipate using temporary punctal plugs during the study that have not been stable within 30 days of Visit 1;

j. Be currently taking any topical ophthalmic prescription (including medications for glaucoma) or over-the-counter (OTC) solutions, artificial tears, gels or scrubs, and cannot discontinue these medications for the duration of the trial (excluding medications allowed for the conduct of the study);

k. Have corrected visual acuity greater than or equal to logMAR +0.7 as assessed by Early Treatment of Diabetic Retinopathy Study (ETDRS) scale in both eyes at Visit 1;

l. Have an uncontrolled systemic disease;

m. Be a woman who is pregnant, nursing or planning a pregnancy;

n. Be unwilling to submit a urine pregnancy test at Visit 1 and Visit 5 (or early termination visit) if of childbearing potential. Non-childbearing potential is defined as a woman who is permanently sterilized (e.g. has had a hysterectomy or bilateral tubal ligation or bilateral oophorectomy), or is post-menopausal (without menses for 12 consecutive months);

o. Be a woman of childbearing potential who is not using an acceptable means of birth control; acceptable methods of contraception include: hormonal oral, implantable, injectable, or transdermal contraceptives; mechanical spermicide in conjunction with a barrier such as a diaphragm or condom; IUD; or surgical sterilization of partner. For non-sexually active females, abstinence may be regarded as an adequate method of birth control; however, if the subject becomes sexually active during the study, she must agree to use adequate birth control as defined above for the remainder of the study;

p. Have a known allergy and/or sensitivity to the study drug or its components;

q. Have a condition or be in a situation which the investigator feels may put the subject at significant risk, may confound the study results, or may interfere significantly with the subject's participation in the study;

r. Be currently enrolled in an investigational drug or device study or have used an investigational drug or device within 30 days of Visit 1;

s. Be currently using any medication known to cause ocular drying (e.g. antihistamines, anti-depressants) or increased lacrimation (e.g. cholinergics) that is not used on a stable dosing regimen for at least 30 days prior to Visit 1;

t. Be receiving systemic corticosteroid therapy (not including inhaled corticosteroids), ophthalmic topical steroids, immunotherapy, or cytotoxic therapy within 14 days of Visit 1 or anticipate such therapy throughout the study period.

u. Be unable or unwilling to follow instructions, including participation in all study assessments and visits.

5.5 Withdrawal Criteria (if applicable)

If at any time during the study the investigator determines that a subject's safety has been compromised, the subject may be withdrawn from the study.

Subjects may withdraw consent from the study at any time.

Sponsor and/or investigator may discontinue any subject for non-compliance or any valid medical reason (see Section 8.6.2).

6 STUDY PARAMETERS

6.1 Efficacy Measures

6.1.1 Primary Efficacy Variables

The following primary endpoints will be tested:

- Change from baseline in ocular discomfort change from pre-CAE[SM] [redacted] to post CAE[SM] [redacted] at Day 29 (Visit 5) using the Ora Calibra™ Ocular Discomfort Scale;

6.1.2 Hierarchical Efficacy Variables

- Change from baseline in inferior corneal staining at Day 29 pre-CAE[SM] [redacted] using the Ora Calibra™ scale.

6.1.3 Secondary Efficacy Variables

- Corneal fluorescein staining (Ora Calibra™ scale) at Visits 3, 4, and 5 (Change from Pre-CAE[SM] to Post-CAE[SM], Pre- and Post-CAE[SM]; [redacted]
- Corneal fluorescein staining (Ora Calibra™ scale) at Visits 3, 4, and 5 (Change from Pre-CAE[SM] to Post-CAE[SM], Pre- and Post-CAE[SM]; [redacted]
- Lissamine green staining (Ora Calibra™ scale) at Visits 3, 4, and 5 (Pre- and Post-CAE[SM]; [redacted];
- Tear film break-up time at Visits 3, 4, and 5 (Pre- and Post-CAE[SM]);
- Ocular Protection Index (OPI 2.0) at Visit 5 (Pre-CAE[SM]);
- Unanesthetized Schirmer's Test at Visit 5 (Pre-CAE[SM]);
- Drop comfort assessment after randomization at Visit 2;
- Ocular Surface Disease Index (OSDI)© at Visits 3, 4, and 5 (Pre-CAE[SM]);
- Ocular discomfort and dry eye symptoms at Visits 3, 4, and 5 (Change from Pre-CAE[SM] to Post-CAE[SM], Pre- and Post-CAE[SM]);
- Ocular discomfort during CAE[SM] at Visits 4 and 5; and
- Daily diary.

6.2 Safety Measures

- Visual acuity (ETDRS) at Visits 1, 2, 3, 4, and 5;

- Slit-lamp biomicroscopy at Visits 1, 2, 3, 4, and 5;
- Corneal Sensitivity at Visits 1 and 5 (Pre-CAE^{SM});
- Adverse event query at Visits 1, 2, 3, 4, and 5;
- Undilated Fundoscopy at Visits 1 and 5; and;
- Intraocular Pressure at Visits 1 and 5.

7 STUDY MATERIALS

7.1 Study Drug(s)

7.1.1 Formulations

Run-In

Placebo Ophthalmic Solution (Vehicle)

Randomized Study Treatments

- 0.1% RGN-259 Ophthalmic Solution
- Placebo Ophthalmic Solution (Vehicle)

7.1.2 Dispensation Schedule

- At the end of Visit 1, qualified subjects will receive run-in (Placebo) for two weeks, dosing QID until Visit 2. Subject will be instructed to dose in the morning, noon, afternoon, and in the evening before bed;

- At the end of Visit 2, qualified subjects will be randomized and the first dose of study drug will be administered in office. A 2 week supply of 0.1% RGN-259 Ophthalmic Solution or Placebo Ophthalmic Solution will be dispensed for dosing QID.

- At Visits 3, 4, and 5 remaining/used study drug will be collected from subjects for drug accountability. At Visit 3, subjects will be redispensed the same kit given to them at Visit 2. At Visit 4, subjects will receive a new kit with an additional 2 week supply of the same test article they were previously randomized to.

- Subjects will be instructed to not use run-in (at Visit 2) or study drug on the day of visits (Visit 3, 4, and 5) prior to the visit.

7.1.3 Instructions for Use

Subjects will be instructed to dose in each eye four times daily (morning, noon, afternoon, and in the evening before bed). Subjects will be

instructed to use a second drop only if the first drop does not fully coat the eye. Subjects will be given detailed instructions on study drug administration, accountability, and storage at each visit.

7.2 Other Study Supplies

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

8 STUDY METHODS AND PROCEDURES

8.1 Subject Entry Procedures

8.1.1 Overview

Subjects as defined by the criteria in sections 5.2, 5.3, and 5.4 will be considered for entry into this study.

8.1.2 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).

8.1.3 Washout Intervals

Prohibited medications, treatments, and activities are outlined in the Exclusion Criteria (Section 5.4).

8.1.4 Procedures for Final Study Entry

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

8.1.5 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. The Interactive Voice/Web Response System (IVRS/IWRS) will be used to account for the stratification factors.

Subjects will be stratified using the worst eye. The worst eye is the eye that meets all of the inclusion criteria. In the case that both eyes are eligible for analysis, the worst eye will be the eye with the largest increase

in ocular discomfort from pre-CAE[SM] [redacted] post-CAE[SM] at Visit 2. If the ocular discomfort symptom increase is the same in both eyes then the worst eye will be the eye with worse (higher) [redacted] staining pre-CAE[SM] at Visit 2. If the i[redacted] staining is the same in both eyes then the right eye will be selected as the worst eye.

Subject's will be stratified by the following factors and cut-offs in the worst eye:

1. Change Pre-CAE[SM] [redacted] Post-CAE[SM] in ocular discomfort (Ora Calibra Ocular™ Discomfort Scale) at Visit 2.

[redacted]

[redacted]

2. Pre-CAE[SM] [redacted] fluorescein staining (Ora Calibra ™ Scale) at Visit 2

[redacted]

[redacted]

Both strata will be entered into the IVRS/IWRS, which will assign the randomization number and the assigned kit number for the patient. The site staff will dispense to the patient the study kit labeled with the corresponding kit number. Both the randomization number and the dispensed study drug kit number will be recorded on the patient's source document and eCRF. A new kit will be dispensed at Visits 2 and 4 based on the subject's randomization. At Visit 3, the subject will be redispensed the Visit 2 kit. The Sponsor, Investigators, and study staff will be masked during the randomization process and throughout the study.

8.2 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 electronic case report form (eCRF) along with the reason the medication was taken.

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

8.2.1 Prohibited Medications/Treatments

Disallowed medications/treatments during the study are outlined in the Exclusion Criteria (Section 5.4).

8.2.2 Escape Medications

No escape medications are required for this study.

8.2.3 Special Diet or Activities

No special diets or activities are required for this study.

8.3 Examination Procedures

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

The following procedures will be performed (see Appendix 2 for description):

Visit 1 (Day -14 ±1): Screening

Pre-CAE℠ Procedures

- Informed consent / HIPAA;
- Demographic data and medical / medication history;
- Urine pregnancy test (for females of childbearing potential);
- Ora Calibra™ Ocular Discomfort;
- Ora Calibra™ Ocular Discomfort & 4-Symptom Questionnaire;
- Ocular Surface Disease Index© (OSDI©);
- Visual Acuity;
- Review of qualification criteria;
- Slit-lamp Biomicroscopy;
- Tear Film Break-up Time (TFBUT)©;
- Fluorescein staining Ora Calibra™;
- Lissamine Green staining Ora Calibra™ ;
- Corneal Sensitivity;
- Unanesthetized Schirmer's Test, to be performed at least 15 minutes after Lissamine Green staining;
- [redacted] CAE℠ exposure;
  - [redacted]

Post- CAE℠ Procedures

- Ora Calibra™ Ocular Discomfort;

- Ora Calibra™ Ocular Discomfort & 4-Symptom Questionnaire;
- Slit-lamp Biomicroscopy;
- TFBUT©;
- Fluorescein staining Ora Calibra™;
- Lissamine Green staining Ora Calibra™;
- Intraocular Pressure;
- Undilated Fundoscopy;
- Review of qualification criteria;
- Adverse event query;
- Dispensation of run-in (RUN-IN) for QID dosing until Visit 2;
    - Subjects will be instructed to dose in each eye four times daily (morning, noon, afternoon, and in the evening before bed);
- Dispensation of diaries and subject instructions;
    - Subjects will be instructed to complete diary symptoms BID prior to drop instillation in the morning and evening before bed until Visit 2;
    - Subjects will be instructed to complete their diary QID to account for each dose of study drug taken;
    - Subjects will be instructed to complete their diary and dose in each eye in the afternoon and before bed on the evening of Visit 1;
    - Subjects will be instructed to complete their diary on the morning of their next visit (Visit 2);
    - Subjects will be instructed to not dose with run-in on the morning of their next visit (Visit 2);
- Qualified subjects will be scheduled for Visit 2.

Visit 2 (Day 1): Confirmation/Baseline

Pre-CAE℠ Procedures

- Collection and review of run-in (RUN-IN) and subject diaries;
- Subject will be asked if he/she dosed with run-in on the morning of the visit; if the subject indicates he/she dosed that morning, he/she should wait at least 4 hours from the time of dosing before efficacy assessments are performed;
- Medical/medication history update;

- Adverse event query;
- Ora Calibra™ Ocular Discomfort;
- Ora Calibra™ Ocular Discomfort & 4-Symptom Questionnaire;
- OSDI©;
- Visual Acuity;
- Review of qualification criteria;
- Slit-lamp Biomicroscopy;
- Ora Calibra™ OPI 2.0;
- TFBUT©;
- Fluorescein staining Ora Calibra™;
- Lissamine Green staining Ora Calibra™;
- Unanesthetized Schirmer's Test, to be performed at least 15 minutes after Lissamine Green staining;
- [redacted] CAE[SM] exposure;
  - [redacted]

Post- CAE[SM] Procedures

- Ora Calibra™ Ocular Discomfort;
- Ora Calibra™ Ocular Discomfort & 4-Symptom questionnaire;
- Slit-lamp Biomicroscopy;
- TFBUT©;
- Fluorescein staining Ora Calibra™;
- Lissamine Green staining Ora Calibra™;
- Adverse Event Query;
- Review of qualification criteria;
- Randomization & Dispensation of study drug kit according to randomization for QID dosing until Visit 4;
- Tear Collection may be performed at least 30-minutes from Lissamine Green staining;
- Subject self-administers first dose of study drug, OU;
- Ora Calibra™ Drop Comfort and Questionnaire;

- Subjects will be instructed to dose in each eye four times daily (morning, noon, afternoon, in the evening before bed);
- Dispensation of diaries;
    - Subjects will be instructed to complete diary symptoms BID prior to drop instillation in the morning and in the evening before bed until Visit 3;
    - Subjects will be instructed to complete their diary QID to account for each dose of study drug taken;
    - Subjects will be instructed to complete their diary and dose in each eye in the afternoon and before bed on the evening of Visit 2;
    - Subjects will be instructed to complete their diary on the morning of their next visit (Visit 3);
    - Subjects will be instructed to not dose with study drug on the morning of their next visit (Visit 3);
- Qualified subjects will be scheduled for Visit 3.

Visit 3 (Day 8 ± 1): 1-Week Follow-Up

- Collection and review of study drug and subject diaries;
- Subject will be asked if he/she dosed with study drug on the morning of the visit; if the subject indicates he/she dosed that morning, he/she should wait at least 4 hours from the time of dosing before efficacy assessments are performed;
- Medical / medication history update;
- Adverse event query;
- Ora Calibra™ Ocular Discomfort;
- Ora Calibra™ Ocular Discomfort & 4-Symptom Questionnaire;
- OSDI©;
- Visual Acuity;
- Slit-lamp Biomicroscopy;
- TFBUT©;
- Fluorescein staining Ora Calibra™;
- Lissamine Green staining Ora Calibra™;
- Unanesthetized Schirmer's Test, to be performed at least 15 minutes after Lissamine Green staining;

- Adverse Event Query;
- Re-dispensation of study drug kit for QID dosing until Visit 4;
- Subject self-administers dose of study drug, OU;
- Ora Calibra™ Drop Comfort and Questionnaire;
- Subjects will be instructed to dose in each eye four times daily (once in the morning, once at noon, once in the afternoon and once in the evening before bed);
- Dispensation of diaries;
    - Subjects will be instructed to complete diary symptoms BID prior to drop instillation in the morning and evening prior to bed until Visit 4;
    - Subjects will be instructed to complete their diary QID to account for each dose of study drug taken;
    - Subjects will be instructed to complete their diary and dose in each eye in the afternoon and before bed on the evening of Visit 3;
    - Subjects will be instructed to complete their diary on the morning of their next visit (Visit 4);
    - Subjects will be instructed to not dose with study drug on the morning of their next visit (Visit 4);
- Subjects will be scheduled for Visit 4.

Visit 4 (Day 15 ± 1): 2-Week Follow-Up

Pre-CAE℠ Procedures

- Collection and review of study drug and subject diaries;
- Subject will be asked if he/she dosed with study drug on the morning of the visit; if the subject indicates he/she dosed that morning, he/she should wait at least 4 hours from the time of dosing before efficacy assessments are performed;
- Medical / medication history update;
- Adverse event query;
- Ora Calibra™ Ocular Discomfort;
- Ora Calibra™ Ocular Discomfort & 4-Symptom Questionnaire;
- OSDI©;
- Visual Acuity;

- Slit-lamp Biomicroscopy;
- TFBUT©;
- Fluorescein staining Ora Calibra™;
- Lissamine Green staining Ora Calibra™;
- Unanesthetized Schirmer's Test, to be performed at least 15 minutes after Lissamine Green staining;
- [redacted] CAE℠ exposure;
    - [redacted];

Post-CAE℠ Procedures

- Ora Calibra™ Ocular Discomfort;
- Ora Calibra™ Ocular Discomfort & 4-Symptom questionnaire;
- Slit-lamp Biomicroscopy;
- TFBUT©;
- Fluorescein staining Ora Calibra™;
- Lissamine Green staining Ora Calibra™;
- Tear Collection may be performed at least 30-minutes from Lissamine Green staining;
- Adverse Event Query;
- Dispensation of study drug kit according to randomization for QID dosing until Visit 5;
- Subject self-administers dose of study drug, OU;
- Ora Calibra™ Drop Comfort and Questionnaire;
- Subjects will be instructed to dose in each eye four times daily (morning, noon, afternoon, and in the evening before bed);
- Dispensation of diaries;
    - Subjects will be instructed to complete diary symptoms BID in the morning and the evening before bed prior to drop instillation until Visit 5;
    - Subjects will be instructed to complete their diary QID to account for each dose of study drug taken;
    - Subjects will be instructed to complete their diary and dose in each eye in the afternoon and before bed on the evening of Visit 4;

- Subjects will be instructed to complete their diary on the morning of their next visit (Visit 5);
- Subjects will be instructed to not dose with study drug on the morning of their next visit (Visit 5);

- Subjects will be scheduled for Visit 5.

Visit 5 (Day 29 ± 2): 4-Week Follow-Up and Study Exit

Pre-CAE^{SM} Procedures

- Collection and review of study drug and subject diaries;
- Subject will be asked if he/she dosed with study drug on the morning of the visit; if the subject indicates he/she dosed that morning, he/she should wait at least 4 hours from the time of dosing before efficacy assessments are performed;
- Medical / medication history update;
- Adverse event query;
- Urine pregnancy test (for females of childbearing potential);
- Ora Calibra™ Ocular Discomfort;
- Ora Calibra™ Ocular Discomfort & 4-Symptom Questionnaire;
- OSDI©;
- Visual Acuity;
- Slit-lamp Biomicroscopy;
- Ora Calibra™ OPI 2.0;
- TFBUT©;
- Fluorescein staining Ora Calibra™;
- Lissamine Green staining Ora Calibra™;
- Corneal Sensitivity;
- Unanesthetized Schirmer's Test, to be performed at least 15 minutes after Lissamine Green staining;
- [redacted] CAE^{SM} exposure;
  - Ora Calibra™ Ocular Discomfort [redacted]

Post-CAE^{SM} Procedures

- Ora Calibra™ Ocular Discomfort;

- Ora Calibra™ Ocular Discomfort & 4-Symptom Questionnaire;
- Slit-lamp Biomicroscopy;
- TFBUT©;
- Fluorescein staining Ora Calibra™;
- Lissamine Green staining Ora Calibra™;
- Tear Collection may be performed at least 30-minutes from Lissamine Green staining;
- Intraocular Pressure;
- Undilated Fundoscopy;
- Adverse event query;
- Study Exit.

Early Termination/Discontinuation

If a subject is discontinued from the study prior to Visit 5 (Day 29 2), then all safety evaluations and Pre-CAE℠ evaluations that are to be performed at Visit 5 (Day 29 2) should be performed on the day of discontinuation (early termination) or at the discretion of the investigator.

Adverse Events (both elicited and observed) and SAEs will be monitored throughout the study. The investigator will promptly review all adverse events (both elicited and observed) for accuracy and completeness. All adverse events will be documented on the appropriate source document and case report form.

If a female reports a pregnancy or has a positive pregnancy test during the study the investigator will notify Ora immediately. The investigator shall request from the subject and/or the subject's physician copies of all related medical reports during the pregnancy and shall document the outcome of the pregnancy. The investigator will retain these reports together with the subject's source documents and will provide a copy of all documentation to Ora.

8.4 Schedule of Visits, Measurements and Dosing

8.4.1 Scheduled Visits

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

8.4.2 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:

- Slit-lamp Biomicroscopy;
- Visual Acuity;
- Intraocular Pressure;
- Pregnancy Test;
- Undilated Fundoscopy;
- Assessment of Adverse Events;
- Assessment of concomitant medications and/or treatments; and
- Any other assessments needed in the judgment of the investigator.

8.5 Compliance with Protocol

Subjects will be instructed on proper use of the subject daily diary and proper instillation and storage of study drug at the end of Visits 1, 2, 3, and 4, and given written instructions. The subject daily diaries and 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.

In the subject daily diary, if more than 20% of Dose Taken boxes are checked "no", left blank, or missing for a diary period, a subject will be deemed non-compliant and a diary deviation will be recorded. If more than 20% of the total diary symptom assessments for that dosing period are missed, these subjects will be deemed non-compliant and a diary symptom assessment deviation will be recorded. These guidelines will be used by the Investigator for determining the subject's necessary compliance for the study and for recording deviations from this compliance.

8.6 Subject Disposition

8.6.1 Completed Subjects

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

8.6.2 Discontinued subjects

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

- adverse events;
- protocol violations;
- administrative reasons (e.g., inability to continue, lost to follow up);
- sponsor termination of study;
- subject choice (e.g. withdrawal of consent); and
- other

Note: In addition, any subject may be discontinued for any sound medical reason at the discretion of the investigator.

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

Discontinued subjects will not be replaced.

8.7 Study Termination

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

8.8 Study Duration

An individual subject's participation will involve 5 visits over approximately a 6 week period (42 days).

8.9 Monitoring and Quality Assurance

During the course of the study a monitor, or designee, will make routine site visits to review protocol compliance, assess study drug accountability, subject safety, 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.

9 ADVERSE EVENTS

9.1 Adverse Event

An adverse event is defined as any untoward medical occurrence associated with the use of a drug in humans, whether or not the event is considered drug-related. An adverse event can therefore be any unfavorable and unintended sign (e.g., an abnormal laboratory finding), symptom, or disease occurring after the subject started dosing with the study drug, without any judgment about causality. Any pre-existing medical condition that worsens after administration of the study drug will also be considered a new adverse event.

If there is a worsening of a medical condition that was present prior to the administration of the study drug, this should also be considered a new adverse event and reported. Any medical condition present prior to the administration of the study drug that remains unchanged or improved should not be recorded as an adverse event at subsequent visits.

Study drug includes the investigational drug under evaluation and any comparator drug, placebo, or any other medications required by the protocol given during any stage of the study.

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

9.1.1 Severity

Severity of an adverse event is defined as a qualitative assessment of the degree of intensity of an adverse event as determined by the investigator or reported to him/her by the patient/subject. The assessment of severity is made irrespective of relationship to study drug 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.

9.1.2 Relationship to Study Drug

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

- *Definite:* When there are good reason and sufficient documentation to demonstrate a direct causal relationship between investigational product and AE;
- *Probable:* When there are good reasons and sufficient documentation to assume a causal relationship in the sense of plausible, conceivable, likely but not necessarily highly probable.
- *Possible:* When there is sufficient information to accept the possibility of a causal relationship in the sense of not impossible and not unlikely, although the connection is uncertain or doubtful, for example; due to missing data or insufficient evidence.
- *None:* When there is sufficient information to accept a lack of a causal relationship, in the sense of impossible and improbable.
- *Unclassified:* When the causal relationship is not assessable for whatever reason due to insufficient evidence, conflicting data or poor documentation.

9.1.3 Expectedness

The expectedness of an adverse event should be determined based upon existing safety information about the study drug using these explanations:

- *Unexpected*: An adverse event that is not listed in the Investigator's brochure or is not listed at the specificity or severity that has been observed.
- *Expected*: An adverse event that is listed in the Investigator's brochure at the specificity and severity that has been observed.
- *Not Applicable*: Any adverse event that is unrelated to the study drug.

Adverse events that are mentioned in the Investigator's brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation are to be considered unexpected.

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

9.2 Serious Adverse Events

An adverse event 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 adverse event;

Note: An adverse event is considered "life-threatening" if, in the view of either the investigator or sponsor, its occurrence places the patient or subject

at immediate risk of death. It does not include an adverse event 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 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 patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

9.3 Procedures for Reporting Adverse Events

All adverse events and their outcomes must be reported to Ora, the study 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 eCRF.

9.3.1 Reporting a Suspected Unexpected Adverse Reaction

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

9.3.2 Reporting a Serious Adverse Event

To ensure subject safety, all serious adverse events, regardless of relationship to the study drug, must be immediately reported. All information relevant to the serious adverse event must be recorded on the appropriate case report forms. The investigator is obligated to pursue and obtain information requested by Ora and/or the sponsor in addition to that information reported on the case report form. All subjects experiencing a serious adverse event must be followed up and the outcome reported.

In the event of a serious adverse event, 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 study 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 study drug; and inform the IRB of the adverse event within their guidelines for reporting serious adverse events.

Contact information for reporting Serious Adverse Events:

Name: Title: Company: Office Telephone: Alternative Telephone:	[redacted]
Office Facsimile: Name: Title: Office Telephone: Mobile Phone: Office Facsimile:	[redacted]

9.4 Procedures for Unmasking of Study Drug

All subjects, investigators, and study personnel involved with the conduct of the study will be masked with regard to treatment assignments. When medically necessary, the investigator may need to determine what treatment arm has been assigned to a subject. When possible (i.e., in non-emergent situations), Ora and/or the study sponsor should be notified before unmasking study drug.

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

The investigator will follow unresolved AEs to resolution until the subject is lost to follow-up or until the AE is otherwise classified. Resolution means the subject has returned to baseline state of health or the Investigator does not expect any further improvement or worsening of the AE. If the patient is lost to follow-up, the Investigator should make 3 reasonable attempts to contact the patient via telephone, post, or certified mail. All follow-up will be documented in the subject's source document. Non-serious AEs identified on the last scheduled contact must be recorded on the AE eCRF with the status noted.

If the Investigator becomes aware of any new information regarding an existing SAE (i.e., resolution, change in condition, or new treatment), a new SAE/Unanticipated Report Form must be completed and faxed to Ora within 24 hours of the site's awareness of the new information. The original SAE form is not to be altered. The report should describe whether the event has resolved or continues and how the event was treated.

10 STATISTICAL HYPOTHESES AND METHODS OF ANALYSES

10.1 Analysis Populations

The following analysis populations will be considered:

- Intent-to-Treat Population The intent-to-treat (ITT) population includes all randomized subjects. The primary analysis will be performed on the ITT population with the Last Observation Carried Forward (LOCF) imputation method for missing values. The ITT population may also be analyzed with observed data only (i.e., without LOCF) and using multiple imputation methods to assess sensitivity. Subjects in the ITT population will be analyzed as randomized.

- Per Protocol Population The per protocol (PP) population includes subjects in the ITT 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 efficacy variables. Subjects in the PP population will be analyzed as randomized.

- Safety Population The safety population includes all 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 ITT population. The primary efficacy analysis will also be performed on the PP population as sensitivity analyses.

10.2 Statistical Hypotheses

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

H_0 : There is no difference between RGN-259 ophthalmic solution (0.1%) and placebo in the change from baseline in ocular discomfort change from pre-CAE℠ [redacted] to post CAE℠ [redacted] at Day 29 (Visit 5) using the Ora Calibra™ Ocular Discomfort Scale.

H : The change from baseline in ocular discomfort change from pre-CAE℠ [redacted] to post CAE℠ [redacted] at Day 29 (Visit 5) using the Ora Calibra™ Ocular Discomfort Scale is less with RGN-259 ophthalmic solution (0.1%) than with placebo.

H_{02}: There is no difference between RGN-259 ophthalmic solution (0.1%) and placebo in the change from baseline in [redacted] staining at Day 29 pre-CAE℠ [redacted] using the Ora Calibra™ scale.

H_2: The change from baseline in [redacted] at Day 29 pre-CAE℠ in [redacted] of subjects with baseline [redacted] using the Ora Calibra™ scale is less with RGN-259 ophthalmic solution (0.1%) than with placebo.

10.3 Sample Size

The primary objective of the study is to demonstrate a statistically significant difference between the active treatment and placebo.

This study is expected to enroll 297 subjects in each of the two treatment arms, for a total of 594 randomized subjects. Assuming a 5% drop out rate, 282 subjects per group are expected to complete the study.

The following formula for the sample size of a two-sample t-test with equal variance is as follows (note that different software packages will yield slightly different results):

$$n = \frac{\left(z_{1-\alpha} + z_{1-\beta}\right)^2 2\sigma^2}{D^2}$$

Where

n the required sample size per treatment group
$z_{-\alpha}$ the normal probability for two-sided α (if α 0.05, then $z_{-\alpha}$ 1.96)
$z_{-\beta}$ the normal probability for power β (if β 0.9, then $z_{-\beta}$ 1.282)
σ the assumed common standard deviation
D the expected treatment difference

With 297 subjects randomized per treatment arm, and a difference between RGN-259 and placebo as low as [redacted] the study will have [redacted] power to detect a treatment difference. This assumes a two-sided test at alpha 0.05 and a common standard deviation (SD) of [redacted] in both treatment arms. Accounting for up to 5% dropouts in the study, 282 subjects randomized per treatment arm, or 564 total subjects, will have 96.5% power to detect a difference for the primary endpoint. Difference and SD estimates are derived from the phase 2b/3 study.

The hierarchical endpoint of the change from baseline in [redacted] staining at Day 29 pre-CAE[SM] is also considered in terms of statistical power. It is assumed that [redacted] ([redacted] completed subjects accounting for 5% dropouts) of the subjects will have a baseline [redacted]. This endpoint will be tested conditionally upon the primary endpoint being successful, thereby maintaining the study-wide type I error rate for both endpoints. With a sample size of [redacted] subjects per treatment arm with a [redacted] ([redacted] of the randomized subjects) the study will have 94% power to detect a difference as low as [redacted] between RGN-259 and placebo. This assumes a two-sided test at alpha 0.05 and a common SD of [redacted] in both treatment arms. To show a range of power using the same assumptions and accounting for fewer subjects with a [redacted], if only [redacted] of subjects ([redacted] completed subjects per treatment arm) have a baseline [redacted] then the study will have [redacted] power to detect a treatment difference between RGN-259 and placebo. Difference and SD estimates are derived from the phase 2b/3 study, [redacted]

10.4 Statistical Analysis

10.4.1 General Considerations

The quantitative variables will be summarized using number of subjects (n), mean, median, standard deviation, minimum and maximum. The qualitative variables will be summarized using counts and percentages.

All summaries will be presented by treatment group. 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 Visit 2. If a measure is taken both pre-CAE^{SM} and post-CAE^{SM}, the baseline will be the time point matched value at Visit 2. For measures from daily subject diaries, baseline is defined as the average of all days during the run-in period, where daily scores are first obtained by averaging the AM and PM scores for that day, as applicable. For changes from pre-CAE^{SM} to post-CAE^{SM} post first treatment, the change from pre-CAE^{SM} to post-CAE^{SM} at Visit 2 will be considered the baseline value.

All primary and secondary analyses will be 2-sided at a significance level of 0.05.

10.4.2 Unit of Analysis

Safety endpoints will be analyzed for both eyes. For efficacy endpoints, the unit of analysis will be 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 worst change in pre- to post-CAE^{SM} ocular discomfort [redacted] at Visit 2. If the increase in ocular discomfort is the same in both eyes then the study eye will be the eye with the largest pre-CAE^{SM} [redacted] fluorescein staining at Visit 2. If the change in ocular discomfort and the pre-CAE^{SM} [redacted] fluorescein staining is the same in both eyes then the right eye will be selected as the study eye.

10.4.3 Missing Data

The primary efficacy analyses will be performed using the Last Observation Carried Forward (LOCF) imputation method for missing values. For the analysis of total corneal fluorescein staining at Day 29 (Visit 5), the last value from the previous visits will be carried forward, matching pre-CAE^{SM} or post-CAE^{SM} time points. A pre-CAE^{SM} time point will never be imputed for a post-CAE^{SM} value, and vice versa.

For the analysis of ocular discomfort scores at Day 29 (Visit 5), the last value from the previous visits will be carried forward, matching pre-CAE^{SM} or post-CAE^{SM} time points similar to the imputation method for total corneal fluorescein staining. A pre-CAE^{SM} time point will never be imputed for a post-CAE^{SM} value, and vice versa.

An analysis using observed data at Day 29 (Visit 5) only will also be performed. Additionally, Markov Chain Monte Carlo (MCMC) multiple imputation methodology will be used to impute missing data for the analyses of the primary efficacy variables.

No secondary efficacy endpoints or safety endpoints will be imputed.

10.4.4 Multiplicity Consideration

To maintain a type I error rate of 5% over the two comparisons of the active treatment group to placebo, a hierarchical testing procedure will be used for the primary endpoints. First, the change from baseline in the change from pre- to post-CAE^{SM} ocular discomfort at Day 29 for 0.1% RGN-259 ophthalmic solution will be tested versus placebo at α 0.05. If there is a significant difference in this discomfort endpoint, then the hierarchical endpoint, the change from baseline in pre-CAE^{SM} [redacted] staining for 0.1 % RGN-259 ophthalmic solution will be tested versus placebo at α 0.05.

For the sign endpoint to be considered successful significance is required for the primary symptom; therefore, the family-wise error rate is maintained at 0.05 through this fixed sequence testing.

10.4.5 Primary Efficacy Analyses

Change from baseline in ocular discomfort change from pre- to post-CAE^{SM} at Day 29 (Visit 5) using the Ora Calibra™ Ocular Discomfort Scale will be calculated as:

(OD[redacted] at Day 29 OD[redacted] at Day 29) (OD[redacted] at Baseline OD[redacted] at Baseline)

Where OD[redacted] Ocular discomfort at [redacted] in CAE^{SM} (pre-CAE^{SM}) and OD[redacted] Ocular discomfort at [redacted] post-CAE^{SM}

With this calculation, a positive change indicates a worsening of dry eye symptoms compared to baseline. In addition, treatment comparisons between active and placebo will be calculated as active placebo, such that a negative result indicates a better score for the active treatment (i.e., the active treatment had a smaller increase in dry eye symptoms during the CAE compared to baseline than the placebo group).

ANCOVA models will be used to compare the change from baseline to Day 29 (Visit 5) in the change from pre-CAE^{SM} to post-CAE^{SM} in ocular discomfort, as measured on the Ora Calibra™ Ocular Discomfort Scale, between 0.1% RGN-259 Ophthalmic Solution and Placebo. The ANCOVA models will include terms for baseline change from pre-CAE^{SM} to post-CAE^{SM} ocular discomfort and study site. In addition, the study site by treatment interaction will be explored in a separate model to evaluate how the treatment effect may differ across study sites. The primary analysis will use LOCF imputation to have a full accounting of the ITT population at the Day 29 visit.

Sensitivity analyses will be performed on the ITT and PP populations using observed data only, and on the ITT population imputing missing data using multiple imputation methods.

10.4.6 Hierarchical Efficacy Analysis

Change from baseline to Visit 5 (Day 29) in [redacted] fluorescein staining (pre-CAE[SM]) is the hierarchical primary endpoint. 0.1% RGN-259 will be compared to placebo [redacted]. It is assumed that [redacted] of enrolled subjects will have [redacted] (also based on Phase 2/3 study).

An ANCOVA model will be used to compare the change from baseline to Day 29 (Visit 5), between 0.1% RGN-259 and placebo. The ANCOVA model will include terms for baseline [redacted] staining and study site. In addition, the study site by treatment interaction will be explored in a separate model to evaluate how the treatment effect may differ across study sites. The primary hierarchical analysis will use LOCF imputation to have a full accounting of the ITT population at the Day 29 visit.

Sensitivity analyses will be performed following the same strategy as the primary endpoint. Observed values at each visit and time point, as well as changes during CAE[SM] and changes from baseline, will also be analyzed to support the primary results.

10.4.7 Secondary Efficacy Analyses

The continuous and ordinal secondary efficacy variables collected at each visit will be summarized descriptively (n, mean, standard deviation, median, min and max), and analyzed with two-sample t-tests comparing each of the active treatment groups to placebo. All visit-based data will be analyzed at each visit and change from baseline. A Wilcoxon rank sum test and an ANCOVA model adjusting for baseline and site will also be assessed where appropriate. No imputation will be performed for secondary efficacy variables.

Corneal fluorescein staining by region and total, lissamine green staining by region, TFBUT, ocular protection index, un-anesthetized Schirmer's test, Drop comfort assessment, OSDI©, ocular discomfort and dry eye symptoms, and changes from baseline in these measures will be analyzed by visit using two-sample t-tests and Wilcoxon rank sum tests, as appropriate.

The worst symptom for each subject will be identified as the symptom with the highest daily average score during the run-in period (Days -14 to -1) as recorded in the subject diary. The worst symptom and each individual symptom will be analyzed using a two-sample t-test. This

analysis will be completed separately for the morning and evening scores as well as the daily average score. Additionally, the average score for each time point (morning, evening before bed and daily average) will also be analyzed separately using a Wilcoxon rank sum test.

10.4.8 Safety Variables

Adverse events will be coded using the MedDRA dictionary. Frequencies and percentages of subjects with treatment-emergent adverse events (TEAEs), serious TEAEs, and TEAEs causing premature discontinuation will be provided by treatment group. An AE is treatment emergent if it occurs or worsens after the first dose of study treatment. Furthermore, frequencies will be given of subjects with TEAEs by system organ class, by system organ class and preferred term, by system organ class, preferred term and maximal severity, by system organ class, preferred term and strongest relationship, and by system organ class, preferred term, maximal severity, and strongest relationship. Separate analyses will be performed for ocular specific and all AEs (including systemic).

Other safety endpoints including visual acuity, slit-lamp biomicroscopy, corneal sensitivity, undilated fundoscopy, and intraocular pressure will be summarized by treatment group and visit using descriptive statistics. Changes or shifts from baseline will also be summarized where appropriate. For assessments performed by eye, study eye and fellow eye will be summarized separately. In addition, shifts from baseline to worst on-treatment value for ocular safety assessments will be summarized.

A full statistical analysis plan (SAP) will be finalized before database lock and unmasking.

10.4.9 Interim Analyses

No interim analyses are planned for this study.

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

This study will be conducted in compliance with the protocol, Good Clinical Practices (GCPs), including the International Conference on Harmonization (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 study drugs in the countries involved will be adhered to.

11.1 Protection of Human Subjects

11.1.1 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 study sponsor and provided in writing by Ora and/or study sponsor prior to the consent process.

11.1.2 Institutional Review Board (IRB) Approval

This study is to be conducted in accordance with Institutional Review Board 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/ERC approved version of the informed consent form will be used.

11.2 Ethical Conduct of the Study

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

11.3 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 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 study 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 study drug may ultimately be marketed, but the subject's identity will not be disclosed in these documents.

11.4 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 Case Report Forms serves as the investigator's record of a subject's study-related data.

11.4.1 Retention of Documentation

All study related correspondence, subject records, consent forms, record of the distribution and use of all study drug and copies of case report forms 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 study drug. 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.

11.5 Labeling, Packaging, Storage, Accountability, and Return or Disposal of Study Drug

11.5.1 Labeling/Packaging

Investigational drug will be packaged and labeled into clinical kits.

For the run-in period, [redacted] pouches will be packaged in a 2-week clinical kit. Each pouch will contain [redacted] single-use vials and provide a sufficient

medication supply for one day. The [redacted] vial in each pouch will be not used by the study subject and returned to the clinical site.

For the treatment period, [redacted] pouches will be packaged in a 2-week clinical kit. Each pouch will contain [redacted] vials and provide a sufficient supply of randomized study drug for one day. The [redacted] vial in each pouch will be not used by the study subject and returned to the clinical site.

11.5.2 Storage of Study Drug

The study drugs must be stored in a secure area accessible only to the investigator and his/her designees. Study drug(s) must be refrigerated (2-8°C, Do Not Freeze), protected from light, and secured at the investigational site in a locked container.

11.5.3 Accountability of Study Drug

The study drugs are 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 study drugs must only be distributed to subjects properly qualified under this protocol to receive study drug. The investigator must keep an accurate accounting of the study drugs by maintaining a detailed inventory. This includes the amount of study drugs received by the site, amount dispensed to subjects, amount returned to the site by the subjects, and the amount returned to the Sponsor upon the completion of the study.

11.5.4 Return or Disposal of Study Drug

All study drugs will be returned to the sponsor or their designee for destruction.

11.6 Recording of Data on Source Documents and Electronic Case Reports Forms (eCRFs)

All subject data will be captured in the subject source documents which will be transcribed in the eCRFs. The investigator is responsible for ensuring that study data is completely and accurately recorded on each subject's eCRF, source documents, and all study-related materials. 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.

11.7 Handling of Biological Specimens

Tear samples will be submitted to a central laboratory and / or analytical laboratory for processing, storage and analysis. All laboratories meet Good Laboratory Practice requirements. Details of sample collection, handling, storage, and shipping procedures are found in an appropriate study procedure manual

11.8 Publications

Authorship and manuscript composition will reflect cooperation among all parties involved in the study. Authorship will be established before writing the manuscript. The study sponsor and Ora will have the final decision regarding the manuscript and publication.

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13 APPENDICES

Appendix 1: Schedule of Visits and Measurements

Procedure	Visit 1 Day -14 ± 1		Visit 2 Day 1		Visit 3 Day8 ± 1	Visit 4 Day15 ± 1		Visit 5 Day 29 ±2	
	Pre CAE[SM]	Post CAE[SM]	Pre CAE[SM]	Post CAE[SM]		Pre CAE[SM]	Post CAE[SM]	Pre CAE[SM]	Post CAE[SM]
Informed Consent / HIPAA	X								
Medical / Medication History and Demographic	X								
Run in Placebo Collection			X						
Study Drug Collection					X	X		X	
Diary Collection			X		X	X		X	
Medical / Medication History Update			X		X	X		X	
Adverse Event Query		X	X	X	X	X	X	X	X
Pregnancy Test	X[1]							X[1]	
Ocular Discomfort Ora, Calibra™ / Dry Eye Symptoms	X	X	X	X	X	X	X	X	X
OSDI© Questionnaire	X		X		X	X		X	
Visual Acuity (ETDRS)	X		X		X	X		X	
Review of Qualification Criteria	X	X	X	X					
Slit lamp Biomicroscopy	X	X	X	X	X	X	X	X	X

Ocular Protection Index (OPI 2.0) Ora Calibra™ Scale			X					X	
TFBUT©	X	X	X	X	X	X	X	X	X
Fluorescein Staining Ora Calibra™ Scale	X	X	X	X	X	X	X	X	X
Lissamine Green Staining Ora Calibra™ Scale	X	X	X	X	X	X	X	X	X
Corneal Sensitivity (Cochet Bonnet)	X							X	
Unanesthetized Schirmer's Test	X		X		X	X		X	
CAE^{SM} Exposure	X		X			X		X	
CAE^{SM} Discomfort Ora Calibra™ Ocular Disc. Scale	X^2		X^2			X^2		X^2	
Tear Collection				X^3			X^3		X^3
Intraocular Pressure		X							X
Undilated Fundus Exam		X							X
Run in Placebo Dispensation		X							
Randomization				X					
Subject Self instillation of study drug				X	X		X		
Ora Calibra™ Drop Comfort Assessment				X	X		X		
Study Drug Dispensation				X	X^4		X		
Diary Dispensation		X		X	X		X		
Exit Subject from Study									X

X^1 For females of childbearing potential, **X**^{2} [redacted] CAE^{SM} exposure, **X**^{3} Tears may be collected after a 30 minute wait period from lissamine instillation, X^4 IP will be collected and re dispensed at visit 3.

Appendix 2: Examination Procedures, Tests, Equipment, and Techniques

The following examination procedures, tests, equipment and techniques are listed in this Appendix:

Visual Acuity Procedures

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

LogMAR Visual Acuity (VA) must be assessed using an ETDRS chart. The procedure used will be consistent with the recommendations provided for using the ETDRS eye chart. Visual Acuity should be evaluated at the beginning of each visit in the study (i.e., prior to slit-lamp examination). Subjects should use most recent correction to attain their best- corrected visual acuity (BCVA).

Equipment

The visual acuity 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 ETDRS Series 2000 Chart 1 & 2, and the right eye (OD) 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/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/she 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 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/she cannot read a letter, he/she should be encouraged to guess. If the subject identifies a letter as one of two letters, he/she should be asked to choose one 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 including in the last line read. This total sum represents the logMAR visual acuity 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 (OS).

In order to provide standardized and well-controlled assessments of visual acuity during the study, all visual acuity 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/her glasses), the reason for the change in correction should be documented.

Note: A clinically significant visual acuity decrease (defined as an increase of 0.22 or greater in logMAR score) from baseline (Visit 1) will be considered an Adverse Event.

Slit-lamp Biomicroscopy

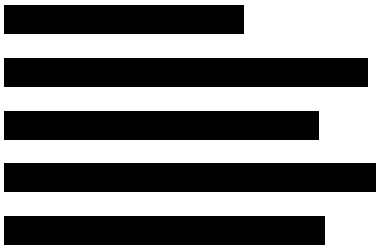
Slit-lamp biomicroscopy will be performed during the study. Observations will be graded as *Normal* or *Abnormal*. Abnormal findings, which are clinically significant, will be described. The following will be examined at each visit:

- **Cornea**
- **Conjunctiva**
- **Anterior Chamber**
- **Iris**
- **Lens**
- **Lid**

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

Ora Calibra™ Ocular Discomfort Scale

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



Ora Calibra™ Ocular Discomfort & 4-Symptom Questionnaire

[redacted]

Ocular Surface and Disease Index (OSDI) ©

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)

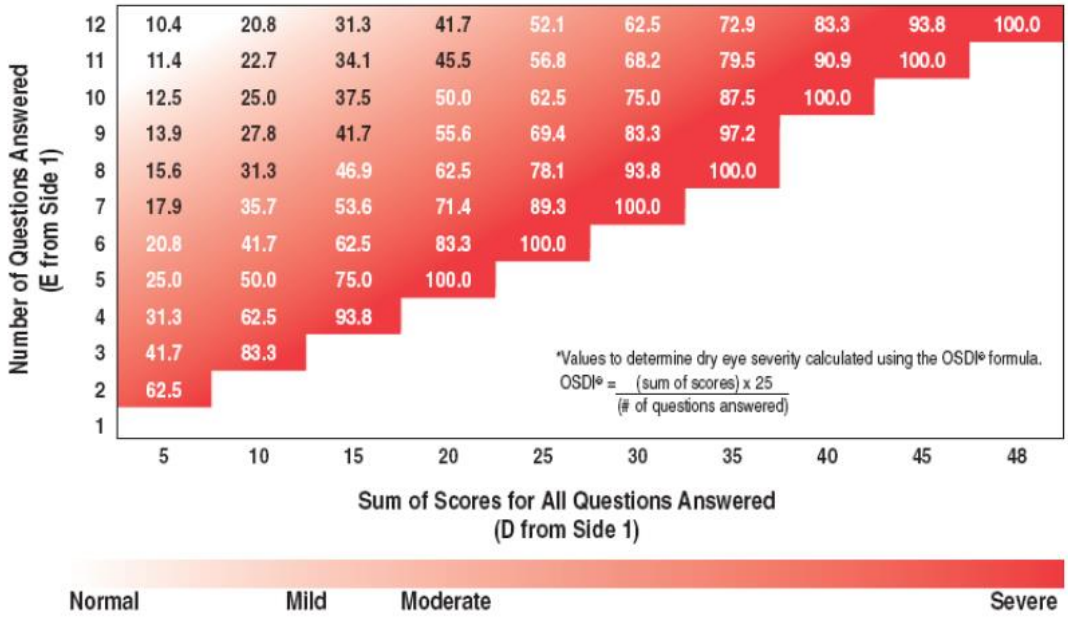
Please turn over the questionnaire to calculate the patient's final OSDI© score.

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

Ora Calibra™ Ocular Protection Index (OPI) 2.0 System

A new, automated methodology will be used to simultaneously measure blink rate, blink pattern, and tear film stability. The system consists of a novel blink pattern recognition and tear film stability device. Together, these tools provide a measure of tear film stability as a percent measure of the area of tear film break up (OPI) and Inter Blink Interval (IBI) caused by corneal drying.

Following instillation of 5 µL of 2% preservative-free sodium fluorescein solution into the inferior conjunctival cul-de-sac, the subject will be asked to blink several times to mix the fluorescein with the tear film. The subject will be instructed to watch television while their tear films are recorded using the EYECAP IM 900 camera system for one minute. A 15 frame per second video of the fluorescent eye under visual task is taken. Subsequently, a computer program will be used to analyze the area of cornea with broken tear film on a frame by frame basis. From this program, IBI and OPI are calculated and produced as assessments of the level of drying of the ocular surface.

Tear Film Break-Up Time (TFBUT) ©

The examiner will instill [redacted] 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 [redacted] 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 for the right eye followed by the left eye. A Wratten #12 yellow filter will be used to enhance the ability to grade TFBUT.

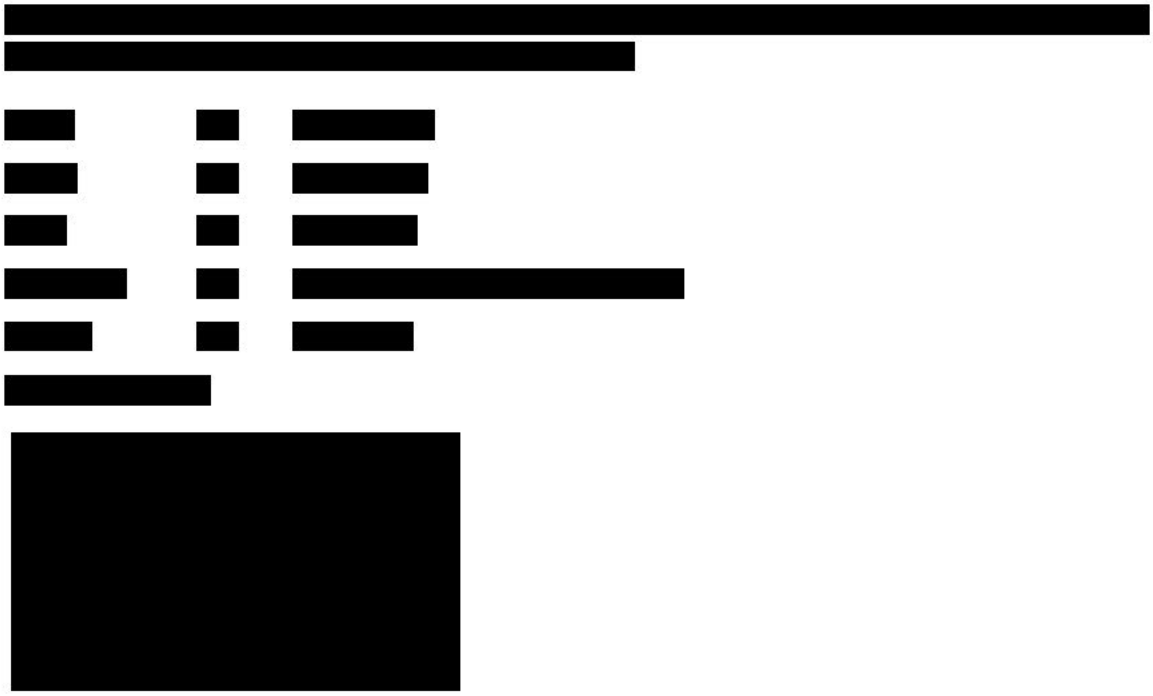
For each eye, two measurements will be taken and averaged unless the two measurements are > 2 seconds apart and are each < 10 seconds, in which case, a third measurement would be taken and the two closest of the three would be averaged.

Fluorescein Staining

The examiner will instill [redacted] solution into the inferior conjunctival cul-de-sac of each eye. In order to achieve maximum fluorescence, the examiner should wait approximately [redacted] 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 Ora Calibra™ Corneal and Conjunctival Staining Scale. Digital images of fluorescein staining may be taken for digital analysis.

Ora Calibra™ Corneal and Conjunctival Staining Scale

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



Lissamine Green Staining

The Investigator will instill [redacted] of lissamine green solution into the inferior conjunctival cul-de-sac and wait approximately [redacted] before evaluating staining. The subject will be instructed to blink several times to distribute the lissamine green. Lissamine may also be applied using lissamine strips. The staining will be graded with the Ora Calibra™ Corneal and Conjunctival Staining Scale. Digital images of lissamine green staining may be taken for digital analysis.

Ora Calibra™ Corneal and Conjunctival Staining Scale

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

[redacted]

[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]

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

Tear Collection

[redacted]

Procedure for Evaluating 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 at Visits 1 and 5, and potentially at an Early Termination Visit. 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.

Procedure for Evaluating Corneal Sensitivity

The aesthesiometer (Cochet Bonnet) is a nylon thread contained in a pen like case that can be extended specific distances from the tip of the case. The following steps will be followed by the examiner for evaluating corneal sensitivity:

1. Remove Cochet Bonnet from box
2. Extend the filament to 60 mm (the filament should not have bends in it if it does, the readings will be incorrect and the filament should not be used)
3. The examiner will inform the subject that a thin delicate plastic filament will be used to test their corneal sensitivity and that they will feel the sensation similar to a strand of hair on their eye. (If the subject is hesitant, a spot on the subject's hand will be cleaned with an alcohol pad and the filament touched to the hand to demonstrate the painless contact)
4. The examiner will instruct the subject to say "Yes" when they feel sensation on their eyeball
5. The examiner will place their free hand on the cheek below the eye being measured to stabilize subject's head position
6. The examiner will position their hand with the device such that the filament is normal (90 degree angle with corneal surface). The device should be between the examiner's index, middle finger, and thumb.
7. The examiner will extend fingers such that the filament applies gentle pressure on the central corneal surface. The examiner will then ask subject if they can perceive the filament touching the central corneal surface.
    a. If the subject responds "Yes", the examiner will ensure the response is valid by performing a sham (not touching the cornea) application and also confirm that response was not due to eyelids touching the filament.
    b. If the subject responds "No", then the filament is shortened the length by 5mm and reapplied to the cornea
    c. If the subject responds "Yes", record length; if the subject responds "No", go to step b.
8. Step 7 is repeated three times.
9. The filament is retracted such that only 10 mm are exposed
10. The tip is wiped with an alcohol pad gently ENSURING that it does NOT bend the filament
11. The alcohol is allowed to dry before reusing device (alcohol can cause corneal abrasions)

Procedure for Conducting Undilated Fundoscopy

An undilated fundoscopy exam will be performed during the study at Visits 1 and 5, and potentially at an Early Termination Visit. Observations will be graded as Normal or Abnormal. Abnormal findings will be described. The following will be examined:

- Vitreous
- Retina
- Macula
- Choroid
- Optic Nerve

Drop Comfort Assessments

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

Subject-Reported Drop Comfort Scale:

Subject-Reported Drop Comfort Questionnaire:

Ora Calibra™ Drop Comfort Questionnaire:

Subject Diary

Subjects will be instructed to complete diary prior to dosing in the morning and evening before bed. Subjects will also indicate if their dose was taken.

Appendix 3: Amendment Summary of Changes

Page	Original Text	Amended Text	Rationale
Throughout document	01Nov2016 Final V1.1	21Jul2017 V2.0	Updated document version
Throughout document		{Grammatical and spelling errors corrected throughout}	Corrections throughout
1	[redacted]	[redacted]	Updated document version
4	[redacted]	[redacted] s	Number of subjects updated
9, 30	• [redacted]	[redacted] .;	Clarification of secondary efficacy measures
10, 30	• [redacted]	[redacted]	Clarification of secondary efficacy measures
10, 31	• [redacted]	[redacted]	Clarification fo safety measures

11 12	[redacted]	[redacted]	Updated per enrollment increase

	[redacted]	[redacted]	
13, 53	[redacted]	[redacted]	Updated for clarification
25	[redacted]	[redacted]	Number of subjects updated
27	[redacted]	[redacted]	Number of subjects updated
49	[redacted]	[redacted]	Updated per enrollment increase
50	[redacted]	[redacted]	Updated per enrollment increase

	[redacted]	[redacted]	
88, 89	[redacted]	[redacted]	Updated document version date

Appendix 4: Sponsor and Ora Approvals

Protocol Title: A Multi-Center, Randomized, Double Masked, Placebo Controlled Clinical Study to Assess the Safety and Efficacy of 0.1% RGN-259 Ophthalmic Solutions for the Treatment of Dry Eye Using the Controlled Adverse Environmental (CAE^{SM}) Model (ARISE-2)

Protocol Number: RGN-259/16-110-0008

Final Date: 21 July 2017

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.

Appendix 5: Investigator's Signature

Protocol Title: A Multi-Center, Randomized, Double Masked, Placebo Controlled Clinical Study to Assess the Safety and Efficacy of 0.1% RGN-259 Ophthalmic Solutions for the Treatment of Dry Eye Using the Controlled Adverse Environmental (CAE^{SM}) Model (ARISE-2)

Protocol Number: RGN-259/16-110-0008

Final Date: 21 July 2017

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:_____

Name: _____

Title: _____

Site: _____

Address: _____

Phone Number: _____