Statistical Analysis Plan

TRIAL FULL TITLE	A Randomized, Multicenter, Double-Masked, Placebo-Controlled Study of the Safety and Efficacy of OmegaD Softgels in the Treatment of Dry Eye Disease
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TRIAL STATISTICIAN	Mark Scott PhD
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TRIAL PRINCIPAL INVESTIGATOR	Charles Slonim, MD
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1 Signatures

I give my approval for the statistical analysis plan (SAP).

Statistician (Author)
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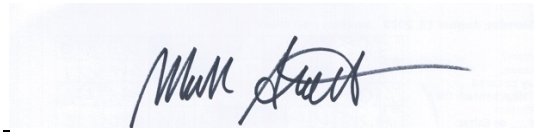
Signature: 

Date: January 16, 2021

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Signature: 

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2 Abbreviations and Definitions

Abbreviation	Explanation
AE	adverse event
eCRF	electronic case report form
FDA	Food and Drug Administration
IP	investigational product
ITT	intent-to-treat
IWRS	Interactive Web Response System
LASIK	laser-assisted in situ keratomileusis
NSAID	nonsteroidal anti-inflammatory drug
OSDI	Ocular Surface Disease Index
OTC	over-the-counter
PP	per protocol
SAE	serious adverse event
SAP	Statistical Analysis Plan
SD	standard deviation
TBUT	tear break-up time
US	United States

3 Introduction

3.1 Preface

Dry eye disease is a common multifactorial ophthalmologic disorder of the tears and ocular surface. Inflammation is an integral component of this disease. Restasis® (cyclosporine ophthalmic emulsion, 0.05%), is a drug that targets the immune system. It was the first Food and Drug Administration (FDA)-approved and most commonly prescribed drug product for patients with dry eye disease. It is indicated for increased tear production in patients whose tear production is presumed to be suppressed due to ocular inflammation associated with keratoconjunctivitis sicca and effectively treats these symptoms in some patients[1]. However, the efficacy of Restasis is considered to be modest, and ocular burning after instillation, the most common adverse reaction, sometimes limits patient compliance and leads to discontinuation of the drug. More recently Xiidra® (lifitegrast) was approved for the treatment of the signs and symptoms of dry eye disease but there remains a clear medical need for additional therapies[2]. Inflammation is a key component of dry eye disease and increasing the systemic levels of omega-3 fatty acids relative to omega-6 levels can mediate immune responses. It is therefore important to evaluate whether omega-3 supplementation can improve dry eye disease signs, symptoms, and associated measures of inflammation.

3.2 Scope of the analyses

These analyses will assess the efficacy and safety of OmegaD softgels in comparison with a placebo control and will be included in the clinical study report.

4 Study Objectives and Endpoints

4.1 Study Objectives

The primary objective of this study is to evaluate the safety and efficacy of once daily dosing of OmegaD softgels in subjects with dry eye disease.

4.2 Endpoints

The two primary efficacy endpoints include mean change from baseline in tear osmolarity in the study eye at Visit 4 (Day 84) and mean change from Screening in Ocular Surface Disease Index (OSDI) score at Visit 4 (Day 84). Secondary efficacy endpoints include mean change from baseline in tear break-up time (TBUT) in the study eye at Visit 4 (Day 84) and mean change from Screening in Schirmer's test (anesthetized) score in the study eye at Visit 4 (Day 84). The proportion of subjects with meibomian gland dysfunction grade of 0 on both meibomian orifice size and telangiectasia scales in the study eye at Visit 4 (Day 84) is an exploratory endpoint.

Safety of OmegaD softgels will be assessed by examination of proportions of subjects experiencing an adverse event during the trial.

5 Study Methods

5.1 General Study Design and Plan

- This is a randomized, multicenter, double-masked, placebo-controlled study.
- Primary efficacy comparison is assessing superiority of OmegaD softgels over placebo softgels.
- Placebo Control with doubling masking of trial materials.
- Randomization is performed by blocks within each center.
- Subjects will be screened within 7 days of baseline. Subjects meeting screening criteria will be evaluated for randomization at the baseline visit (Day 1). Randomized subjects are to receive trial treatments for 84 days with an interim clinic visit at Day 42.

5.1.1 Inclusion-Exclusion Criteria

5.1.1.1 Inclusion

- Subjects age ≥ 18 years and ≤ 90 years on the date of informed consent.
- All subjects must provide signed written consent prior to participation in any study-related procedures.
- At least moderate ocular surface disease as measured by an OSDI score ≥ 20 at Screening.
- Clinical diagnosis of dry eye disease supported by global clinical assessment.
- Presence of tear osmolarity in at least one eye ≥ 312 mOsm/L at both Screening and Baseline.
- Schirmer's test score (anesthetized) ≥ 5 mm in both eyes at Screening.
- TBUT ≤ 7 seconds in both eyes at Screening.
- Presence of meibomian gland dysfunction as defined by a grade of 1 or 2 on the meibomian orifice size scale in at least one eye at both Screening and Baseline. The qualifying osmolarity level and meibomian orifice size grade must be present in the same eye at both Screening and Baseline if only one eye qualifies.

5.1.1.2 Exclusion

- Any previous reconstructive or cosmetic eyelid surgery that may, in the Investigator's opinion, affect the normal function of the lids (eg, blepharoplasty, ptosis repair, entropion/ectropion repair) that could affect study parameters/assessments.
- Cataract extraction, with or without minimally invasive glaucoma surgery (eg, iStent®), within 90 days prior to Screening.
- Any previous invasive glaucoma surgery (eg, trabeculectomy, shunts, valves) and/or corneal surgery (eg, penetrating keratoplasty, lamellar keratoplasty, Descemet's stripping endothelial keratoplasty [DSEK]).
- Lid scrubs with over-the-counter (OTC) products (eg, OCuSOFT® lid scrub, SteriLid®, baby shampoo, etc.) and/or warm compresses within 14 days prior to Screening and throughout the study period.
- Prescription and OTC ophthalmic mast cell stabilizers and antihistamines within 21 days prior to

Screening and throughout the study period (systemic mast cell stabilizers are allowed, and systemic antihistamines are permitted with certain restrictions.

- Chronic daily use (>7 consecutive days at the recommended dosing frequency) of systemic narcotics for any chronic pain syndrome (e.g. fibromyalgia, rheumatoid arthritis, etc.) during the study period. Short-term, as needed dosing of a systemic narcotic for ≤ 72 hours is allowed, but not at Screening or on the day of the study visit.
- Allergy to fish oil or mineral oil (component of placebo softgels) or any component of the softgel material.
- Clinically significant eyelid deformity or eyelid movement disorder that is caused by conditions such as notch deformity, incomplete lid closure, entropion, ectropion, hordeolum, or chalazion.
- Active or anticipated seasonal and/or perennial allergic conjunctivitis or rhinitis.
- Previous ocular disease leaving sequelae or requiring current topical eye therapy other than for dry eye disease, including, but not limited to, active corneal or conjunctival infection or inflammation of the eye and ocular surface scarring.
- History or presence of abnormal nasolacrimal drainage.
- Laser-assisted in situ keratomileusis (LASIK) or photorefractive keratectomy (PRK) performed within one year prior to Screening and throughout the study period.
- Ophthalmic artificial tear drop use within 2 hours prior to any study visit. Any OTC artificial tear (preserved or unpreserved) should be continued at the same frequency and with no change in drop brand.
- Contact lens wear within 12 hours prior to Screening or any study visit; subjects determined to have worn contact lenses within 12 hours must be rescheduled.
- History of cauterization of the punctum or existing silicone punctal plug(s); history of silicone plug removal or collagen plug insertion or removal within 12 months prior to Screening and throughout the study period.
- Started or changed the dose of systemic medications known to affect tear production (including immunomodulators, tricyclic antidepressants, diuretics, and corticosteroids) within 30 days prior to Screening and throughout the study period. A short (≤ 72 hour) course of a systemic medications that affect tear production (including immunomodulators, tricyclic antidepressants, diuretics, and corticosteroids) or systemic antihistamines is allowed but not within 30 days of Screening or on the day before or the day of any other study visit. Any chronic use of systemic antihistamines within 30 days prior to Screening and throughout the study period is prohibited.
- Use of any topical prescription ophthalmic medications (including cyclosporine [Restasis®, Cequa®] or topical lifitegrast [Xiidra®], steroids, nonsteroidal anti-inflammatory drugs [NSAIDs], anti-glaucoma medications, anti-microbials), topical macrolides, or oral nutraceuticals (fish, flax, black currant seed oils, etc.) within 21 days prior to Screening and throughout the study period.
- Use of oral tetracyclines or oral macrolides within 21 days prior to Screening and throughout the study period; use of isotretinoin (Accutane®) within 90 days prior to Screening and throughout the study period.
- Chronic daily use (defined as > 7 consecutive days at the recommended dosing frequency) of

oral NSAIDs during the study period. ANY use of oral NSAIDs during the study period must be discussed with the Medical Monitor. Aspirin of any dosage is permitted.

- Women of childbearing potential who are pregnant, nursing, planning a pregnancy, or not using a medically acceptable form of birth control. Acceptable methods include the use of at least one of the following: intrauterine device (IUD), hormonal (oral, injection, patch, implant, ring), barrier with spermicide (condom, diaphragm), or abstinence. An adult woman is considered to be of childbearing potential unless she is 1 year postmenopausal or post-surgical hysterectomy. All women of childbearing potential, including those with post-tubal ligation, must have a negative urine pregnancy test result at Visit 1 (Screening), Visit 2 (Baseline), and Visit 4 (Day 84) examinations and must intend to not become pregnant during the study.
- Participation in any drug or device clinical investigation within 30 days prior to entry into this study and/or during the period of study participation.

5.2 Randomization and Masking

An Interactive Web Response System (IWRS) assigned a kit number to subjects qualifying for randomization. Subjects were randomized in a 1:1 ratio (OmegaD to placebo). A randomized block design (balanced blocks within the Clinical Site) was used.

Subjects meeting eligibility criteria at Screening and at Baseline were randomly assigned to trial therapy at the Baseline Visit. Clinical sites utilized the IWRS to assign kits to subjects. The investigational product (IP) kit randomization number was recorded in the subject's electronic case report form (eCRF).

OmegaD softgels are identical in appearance to placebo softgels.

5.3 Study Assessments

	Visit 1/(Screening)	Visit 2(Baseline)	Visit 3	Visit 4
	(Day -7 to Day -1)	Day 1	Day 42	Day 84
Obtain written informed consent	x			
Demographics	x			
Medical and ocular histories	x			
Concomitant medication history (review)	x	x	x	x
Urine pregnancy test (women of childbearing potential only)	x	x		x
OSDI	x		x	x
Tear osmolarity test	x	x		x
Meibomian gland dysfunction grading	x	x		x

	Visit 1/(Screening)	Visit 2(Baseline)	Visit 3	Visit 4
	(Day -7 to Day -1)	Day 1	Day 42	Day 84
Slit lamp examination	x	x	x	x
TBUT	x			x
Schirmer's test (anesthetized)	x			x
Review inclusion/exclusion criteria	x	x*		
AE assessment	x	x	x	x
Randomization		x		
HS-Omega-3 Index Test (fingerstick blood sample)		x		x
Dispense and document dispensing of IP and IP diary		x	x	
Collect unused IP and IP diary			x	x
Conduct IP accountability and review IP diary			x	x
Assess compliance with dosing of any vitamins with respect to study medication.				x

Note(*)

- Subjects must have tear osmolarity ≥ 312 mOsm/L and meibomian gland dysfunction grade as defined by a grade 1 or 2 on the meibomian orifice size scale in at least one eye at both Screening and Baseline. The qualifying osmolarity level and meibomian orifice size grade must be present in the same eye at both Screening and Baseline if only one eye qualifies.
- TBUT ≤ 7 seconds in both eyes at Screening (TBUT is not conducted at Baseline)
- Schirmer's test score (anesthetized) at Screening must be ≥ 5 mm in both eyes (Schirmer's test is not conducted at Baseline)
- OSDI ≥ 20 points at Screening

The occurrence of interim visits necessitating efficacy assessments is unlikely. Subjects having efficacy data collected outside these time windows will be considered for inclusion in the per protocol population.

Visit (target day)	Lower bound (days)	Upper bound (days)
Baseline (0)	N/A	N/A
Day 42	35	49
Day 84	77	91

Tear osmolarity and OSDI are the primary efficacy assessments. Secondary efficacy endpoints include TBUT and Schirmer's Test. The study eye will be the worse eye at Baseline as defined by higher tear osmolarity score; if both eyes score equally on tear osmolarity, the eye with the lower TBUT score will be chosen, and if still equal, the right eye will be the study eye.

Tear osmolarity will be measured at Screening, Baseline and at Day 84. Tear osmolarity will be assessed using the TearLab Osmolarity Test. Subjects will require tear osmolarity values ≥ 312 mOsm/L in the study eye at both Screening and Baseline. Subjects must have tear osmolarity ≥ 312 mOsm/L and meibomian gland dysfunction grade as defined by a grade 1 or 2 on the meibomian orifice size scale in

at least one eye at both Screening and Baseline. The qualifying osmolarity level and meibomian orifice size grade must be present in the same eye at both Screening and Baseline if only one eye qualifies.

OSDI will be measured at Screening, Day 42 and Day 84. OSDI is assessed on a scale of 0 to 100, with higher scores representing greater disability. The OSDI is a valid and reliable instrument for measuring dry eye disease severity (normal, mild to moderate, and severe) and effect on vision-related function. At screening subjects must have OSDI scores ≥20. This represents at least moderate dry eye disease. OSDI at screening was the pretherapy assessment and will be used as the Baseline value. OSDI was an eligibility criterium and so the screening visit was taken as the baseline value to prevent any issues regarding learning that can occur from repeat testing in a short space of time. It was recognized that OSDI could fluctuate day-to-day. As potential fluctuations were prior to randomization any change would not introduce bias.

TBUT is determined by measuring the interval between instillation of topical fluorescein and appearance of the first dry spots on the cornea. Three consecutive measurements are taken, and the time is averaged to obtain the TBUT. A TBUT value of less than 10 seconds is considered abnormal. TBUT will be measured at Screening and at Day 84.

Schirmer's test determines whether a subject's eye produces enough tears to keep their eye moist and healthy. To conduct a Schirmer's test, filter paper is applied inside the lower eyelid of both eyes and the subject closes their eyes. After 5 minutes, filter paper is removed. How far the tears have travelled on the paper is the measurement. In general, the smaller the amount of moisture on the paper, the fewer tears that person has produced. Results from Schirmer's test will be available at Screening and at Day 84.

6 Sample Size

A total of 150 subjects per group is planned. No interim analyses are contemplated. The target recruitment of 300 randomized subjects was increased to 320 to account for a number of subjects not being able to attend visits due and hence being dropped due to COVID-19.

Power considerations for each of the primary endpoints are described below.
Tear osmolarity: In the initial placebo-controlled trial of OmegaD (OmegaD-2016-001[3]), the change from baseline in tear osmolarity was -13 (standard deviation [SD]=22). Two dry eye natural history studies (Smith, data on file[4] and Sullivan, 2012[5]) suggest that changes in tear osmolarity can become less or more severe. The former trial reported a mean decrease of 6 mOsm/L, whereas the latter reported a mean increase of approximately 20 mOsm/L. A sample size of 150 in each group will have 95% power to detect a difference in change in tear osmolarity means of 8 mOsm/L (-13 mOsm/L for OmegaD and -5 mOsm/L for placebo), assuming that the common SD of change is 19, using a two-group t-test with an a=0.05 two-sided significance level.
OSDI: As OSDI is a subject-reported outcome, some degree of improvement is expected from trial participation. In study OmegaD-2016-001, the change from baseline in OSDI was -13 points (SD=20). In the natural history studies described above, changes from baseline in OSDI were -7 and -10 points (SD≈16). A sample size of 150 in each group will have approximately 95% power to detect a difference in

change in mean OSDI scores of 7 points (-13-point change for OmegaD and -6-point change for placebo), assuming that the common SD of change in OSDI is 17.0, using a two-group t-test with an a=0.05 two-sided significance level.

Although not a primary endpoint, sample size calculations for TBUT are included. TBUT: In study OmegaD-2016-001, the change from baseline in TBUT was 1.4 seconds (SD=2.8). In the natural history studies described above, changes from baseline in TBUT were 0.4 and 0 seconds (SD≈1.9). A sample size of 150 in each group will have approximately 95% power to detect a difference in change in mean TBUT of 1 second (1.4-second change for OmegaD and 0.4-second change for placebo), assuming that the common SD of change in TBUT is 2.4, using a two-group t-test with an a=0.05 two-sided significance level.

7 General Analysis Considerations

7.1 Timing of Analyses

The statistical analysis will be conducted after the data base has been locked. This will include all data on all randomized subjects with completed follow-up.

7.2 Analysis Populations

7.2.1 Full Analysis Population (or Intention to Treat or Modified Intention to Treat)

- All subjects who were randomized and received any IP.

7.2.2 Per Protocol Population

- All subjects meeting protocol entry criteria, compliant with protocol procedures and investigational product and did not receive prohibited medications thought to affect objective and subjective dry eye measurements. (See Section 7.2 of protocol). Early terminations include subject withdrawing due to Adverse Events, COVID-19 (including subjects without data at Visit 4 and those having Visit 4 assessments telephonically), Subject Withdrawal, Lost to Follow-up and Efficacy Assessments greater than 7 days from last IP dose.
- This population will be determined prior to unmasking of the trial.

7.2.3 Safety Population

- All subjects who received at least one dose of IP

The primary efficacy analysis is based on the full analysis population. The safety assessment will use data from the safety population. Supportive analysis will use the Per Protocol Population.

7.3 Covariates and Subgroups

The primary analysis will use a statistical model that adjusts for trial center as well as the pre-randomization value of the efficacy variable. The effects of center will be examined and potential interactions between centers and IP.

15 Centers with 20 subjects per center are planned. In the event of underperformance (<6 per center) in recruitment among a number of centers, the underperforming centers will be pooled. The strategy for pooling will consider location of the center (academic or clinic) and geography. The pooling will be decided prior to unmasking of the data.

There are no *a priori* subgroups considered for inclusion in the statistical analyses. However, treatment outcomes by gender and age will be displayed.

7.4 Missing Data

The protocol requires that, for subject discontinuation before Visit 4 (Day 84), every effort should be made to keep the subject in the study and conduct all study visits as scheduled or, failing that, to perform all Visit 4 (Day 84) procedures at the visit the subject is discontinued. Also, due to COVID-19, subjects nearing the Day 84 assessment, but not allowed to attend clinic, were allowed to continue IP until a final visit could occur. The visit times may be well over the times in the nominal visit window but data from these visits will be included provided that the subject continued to dose IP and was dosing IP for at least 7 days prior to their V4 visit. For these subjects those data will not be considered missing and used in the primary analyses for the Full Analysis Population.

Efficacy data (osmolarity and/or OSDI) for subjects receiving IP may be missing for Day 84 (e.g. failure to return, Adverse Event). A priori assumptions are that these will be few. The missing data handling method for the primary endpoints, based on the population of randomized and treated subjects, will be as follows: Regression and propensity scores methods of multiple imputation will be used for missing OSDI and tear osmolarity scores at Visit 4 (week 12). As data will only be missing at visit 4, monotone missingness will be assumed. OSDI and Total Osmolarity will be assessed separately.

The PROC MI procedure in SAS will be used. In addition to the regression method, propensity scores will also be employed. The output of interest from PROC MI is a data set containing multiple repetitions of the original data set, along with the newly imputed values. Each of these output datasets will be analyzed using the statistical model that adjusts for trial center as well as the pre-randomization value of the efficacy variable. The MIANALYZE procedure in SAS will be used to combine the results from output datasets from PROC MI.

The model structure in SAS for the OSDI endpoint will be as follows for the regression and propensity scores (similar models for Total Osmolarity will be generated):

```
DATA OSDI_CH; set omegad.osdi_CH; keep trt subject center osdi_v1
osdi_v4 trt;
proc mi data=osdi_CH seed=501213 nimpute=5  out=OSDI_outreg;
class center trt;
monotone reg (OSDI_v4=osdi_v1 center trt);
var center trt OSDI_v1 OSDI_v4;
run;
proc mi data=osdi_CH  seed=501213 nimpute=5  out=OSDI_outprop;
class center trt;
monotone propensity (OSDI_v4=osdi_v1 center trt);
var center trt OSDI_v1 OSDI_v4;
run;
```

Here OSDI_V1 and OSDI_V4 are OSDI values at visits 1 and 4 respectively; trt and center are classification variables for trt and center. OMEGAD.osdi_CH will be the final analysis data set for OSDI.

As a sensitivity check these outcomes will be compared to the results using a last value carried forward approach on the Intention-to-Treat Population and results from the per-protocol population. Differences in approaches will be assessed and reported.

A detailed table will be provided listing subjects screened, attending baseline, randomized and randomized and completed to describe the extent of missing data.

7.5 Interim Analyses and Data Monitoring (as applicable)

No Interim Analysis is planned.

7.6 Multiple Testing

The primary efficacy endpoints (Mean change from baseline in tear osmolarity in the study eye at Day 84 and Mean change from Screening in OSDI score Day 84) comprise a set of hypotheses that will be tested in a hierarchical fashion using Hochberg's procedure. The Hochberg procedure is a step-up procedure. The procedure starts with the largest p-value, which is compared to the largest endpoint-specific critical value ($\alpha=0.05$). If the largest p-value is less 0.05, then the method concludes that there are significant treatment effects for all endpoints.

The differences between the 2 treatment groups for these endpoints will be tested with a significance level of 0.05. In order to control the Type I error rate these 2 endpoints will be tested sequentially in the method described above. For instance, if significance level for the null hypothesis for the tear osmolarity endpoint is the largest and is less than 0.05, OSDI endpoint will also declared significant at $P \leq 0.05$.

8 Summary of Study Data

All continuous variables will be summarized using the following descriptive statistics: n (non-missing sample size), mean, SD, median, maximum and minimum. The frequency and percentages (based on the non-missing sample size) of observed levels will be reported for all categorical measures. In general, all data will be listed, sorted by center, treatment and subject, and when appropriate by visit number within subject. All summary tables will be structured with a column for each treatment in the order (Placebo, OmegaD) and will be annotated with the total population size relevant to that table/treatment, including any missing observations.

8.1 Subject Disposition

A CONSORT diagram will be included delineating subjects screened, randomized and followed will be included.

8.2 Derived variables

Changes from Baseline are the derived variables.

8.3 Protocol Deviations

From Section 7.2 in the protocol the following are deviations that may be associated with subject withdrawal during the post randomization period as they may affect efficacy assessments.

- Use of Lid scrubs with over-the-counter products and/or warm compresses.
- Chronic daily use of systemic narcotics for any chronic pain syndrome
- LASIK or photorefractive keratectomy
- Ophthalmic artificial tear drop use within 2 hours prior to any study visit.
- Contact lens wear within 12 hours of any study visit.
- Cauterization of the punctum or existing silicone punctal plug(s); silicone plug removal or collagen plug insertion or removal
- Started or changed the dose of systemic medications known to affect tear production
- Any chronic use of systemic antihistamines
- Use of any topical prescription ophthalmic medications, topical macrolides, or oral nutraceuticals (fish, flax, black currant seed oils, etc.)
- Use of oral tetracyclines or oral macrolides; use of isotretinoin.
- Chronic daily use (defined as > 7 consecutive days at the recommended dosing frequency) of oral NSAIDs
- Becoming Pregnant.
- Participation in any other drug or device clinical investigation.
- Lack of compliance with respect to timing of dosing of vitamins relative to investigational product.

Subjects with these deviations will be examined and data may be excluded from the per-protocol analysis.

8.4 Demographic and Baseline Variables

Demographic variables include age, gender and clinical site.

8.5 Concurrent Illnesses and Medical Conditions

Medical History and Ocular History are coded with MedDRA V22.1. Concomitant Medications are coded with WHO Drug v. September 2019.

8.6 Treatment Compliance

Compliance will be assessed as the percent of Soft-Gels returned versus dispensed. For those subjects delaying clinic visits due to COVID-19, additional IP was provided as needed.

9 Efficacy Analyses

All efficacy variables will be listed by subject within study center. Data will be summarized by treatment group. N, Mean, Median, SD, Minimum and Maximum will summarize continuous efficacy

variables, whereas number and percent will summarize categorical efficacy variables.

As per E9 Statistical Principles for Clinical Trials, the main treatment effect will be estimated using Model (1) that allows for center differences but does not include a term for treatment-by-center interaction. In the presence of a significant treatment effect, heterogeneity of the treatment effect will be explored by adding treatment by center interaction terms (Model (2)). If no statistically significant interaction is seen, then the estimated treatment effect from Model (1) will be provided. If an interaction between treatment and center is present the nature of the interactions will be explored (quantitative or qualitative) and reported.

Model (1): $y_{ijk}=\mu + c_i + t_j + \xi_{ijk}$, where:

c_i is fixed effect of the ith center

t_j is the fixed effect of the jth treatment

y_{ijk} is the response of the kth subject in jth treatment group within the ith center

ξ_{ijk} is the random measurement error in observing y_{ijk}

μ is a constant effect common to all responses

i=1, . . . , c, j=1 or 2 and k=1, . . . , $n_{ij.}$

Model (2): $y_{ijk}=\mu + c_i + t_j + (ct)_{ij} + \xi_{ijk}$ where $(ct)_{ij}$ is the fixed interaction effect between the ith center and jth treatment.

Analyses will be using the SAS system.

9.1 Primary Efficacy Analysis

The null and alternative hypotheses for this model are H_0: $\alpha=0$ versus $\alpha \neq 0$. Treatment will be tested at the 2-sided 5% significance level. A test for interaction will be performed if the overall effect for investigational product in the model for main effects is significant. In the event of a lack of interaction between center and investigational product the results from the main effect model will be reported (IP and Center). If a beneficial treatment effect is observed for both primary variables (OSDI/tear osmolarity) then OmegaD would be declared effective in dry eye disease.

Box plots will be used to check for normality prior to analysis. Transformations may be used to allow for conformance to normality. Data from previously reported clinical trials in dry eye disease, examining changes in both OSDI and tear osmolarity, were used in estimating sample sizes. Methods sections and results from publications examining these outcomes reflect use of data without need for transformations to achieve normality. However, in the event that normality is not observed, the van Elteren test will be employed in the event of nonnormal distributions. The van Elteren test is an extension of the Wilcoxon rank sum test for detecting differences in the distributions of the response of interest between groups in a stratified experiment.

Statistical tests will be two-sided. Methods for handling missing data are described in Section 8.4.

All assumptions for regression models will be assessed by viewing plots of the residual values.

9.2 Secondary Efficacy Analyses

Slit lamp examinations, TBUT and Schirmer's test will be analyzed as secondary endpoints using the model above on both the per-protocol and intention-to-treat populations. Results from the secondary endpoints will be reported. No formal inferences are planned for secondary endpoints.

9.3 Exploratory Efficacy Analyses

Meibomian gland dysfunction grading will be analyzed as an exploratory endpoint using the model above.

10 Safety Analyses

Adverse event (AE) data will be listed by subject within study center and IP. Tables of proportions of AEs by IP within body system, severity and relationship to IP will be provided.

Tables of All-Cause Mortality and Serious Adverse Events (SAE) will also be included.

No formal statistical analyses of AE data are planned.

10.1 Extent of Exposure

Exposure in days will be summarized by IP using N, Mean, Median, SD, Minimum and Maximum.

10.2 Adverse Events

All AEs will be reported and summarized as proportions with event by IP.

10.3 Deaths, Serious Adverse Events and other Significant Adverse Events

These events, if any, will be tabulated.

10.4 Pregnancies

Pregnancies during the study will be tabulated

10.5 Clinical Laboratory Evaluations

Not Collected.

10.6 Prior and Concurrent Medications

Medications received prior and during the study will be summarized.

10.7 Other Safety Measures

N/A

11 Pharmacokinetics (N/A)

N/A

12 Other Analyses

N/A

13 Reporting Conventions

P-values ≥0.001 will be reported to 3 decimal places; p-values less than 0.001 will be reported as "<0.001". The mean, SD, and any other statistics other than quantiles, will be reported to one decimal place greater than the original data. Quantiles, such as median, or minimum and maximum will use the same number of decimal places as the original data. Estimated parameters, not on the same scale as raw observations (e.g. regression coefficients) will be reported to 3 significant figures.

14 Quality Assurance of Statistical Programming (As Applicable)

The SAS system will be used to created analysis data sets, statistical analyses and summary tables and listings.

Any population to be used in a table, analysis or graphical display will be explicitly set at the start of a block of code that computes the output.

Any outputs will have the

- date and time included
- the name of the code file that produced the analysis
- the author

At the start of any code file there will be a set of comments that give

- the author
- the date and time of writing
- references to inputs and outputs
- reference to any parent code file that runs the child code file

15 Summary of Changes to the Protocol and/or SAP

As of this date no changes to the protocol have been made that materially affect the statistical methods section in the protocol.

16 References

[1] Restasis® (cyclosporine ophthalmic emulsion, 0.05%) US Prescribing Information.
[2] XIIDRA® (lifitegrast ophthalmic solution), US Prescribing Information, Initial U.S. Approval: 2016
[3]Study of the Safety and Efficacy of OmegaD Softgels in the Treatment of Dry Eye Disease: ClinicalTrials.gov Identifier: NCT02980224
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