Sponsor:	Ocular Therapeutix, Inc.		
Protocol:	OTX-DED-2020-201		
Document Version No.:	V0.4V1.0	**Document Date:**	11NOV2021

A randomized, double-masked, vehicle-controlled, phase 2 study to evaluate the efficacy and safety of OTX-DED (dexamethasone intracanalicular ophthalmic insert) for the short-term treatment of signs and symptoms of dry eye disease (DED)

Protocol Number: **(Version Date)**	**OTX-DED-2020-201** (V04 16 June 2021)
Name of Test Drug:	OTX-DED
Phase:	2
Methodology:	**Randomized, double-masked, vehicle-controlled**
Sponsor:	Ocular Therapeutix, Inc. 24 Crosby Drive Bedford, MA 01730 USA
Sponsor Representative:	Alet Van Beek Senior Director, Clinical Operations
Document Date:	11NOV2021
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Sponsor:	Ocular Therapeutix, Inc.		
Protocol:	OTX-DED-2020-201		
Document Version No.:	V1.0	**Document Date:**	11NOV2021

SIGNATURE PAGE

Protocol Title:	A randomized, double-masked, vehicle-controlled, phase 2 study to evaluate the efficacy and safety of OTX-DED (dexamethasone intracanalicular ophthalmic insert) for the short-term treatment of signs and symptoms of dry eye disease (DED)
Sponsor:	Ocular Therapeutix, Inc. 24 Crosby Drive Bedford, MA 01730 USA
Protocol Number:	OTX-DED-2020-201
Document Date/Version:	11NOV2021/V1.0
Cytel, Inc. Author:	Signature: Akaberi Date: Nov 11th 2021

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Sponsor:	Ocular Therapeutix, Inc.		
Protocol:	OTX-DED-2020-201		
Document Version No.:	V1.0	**Document Date:**	11NOV2021

Sponsor Approval

By signing this document, I acknowledge that I have read the document and approve of the planned statistical analyses described herein. I agree that the planned statistical analyses are appropriate for this study, are in accordance with the study objectives, and are consistent with the statistical methodology described in the protocol, clinical development plan, and all applicable regulatory guidance's and guidelines.

I have discussed any questions I have regarding the contents of this document with the biostatistical author.

I also understand that any subsequent changes to the planned statistical analyses, as described herein, may have a regulatory impact and/or result in timeline adjustments. All changes to the planned analyses will be described in the clinical study report (CSR).

Sponsor Signatories:

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ABBREVIATIONS

Abbreviation	Definition
AE	Adverse Event/Experience
AT	Artificial Tears
BCVA	Best Corrected Visual Acuity
C	Celsius
CD	Compact Disc
CCLRU	Cornea and Contact Lens Research Unit
CFB	Change from Baseline
CFR	Code of Federal Regulations
CFS	Corneal Fluorescein Staining
CRF	Case Report Form
CRO	Contract Research Organization
CV	Curriculum Vitae
DED	Dry Eye Disease
E	Electronic
EDC	Electronic Data Capture
ETDRS	Early Treatment of Diabetic Retinopathy Study
FCS	Fluorescein Corneal Scoring
FCS MI	Fully Conditional Specification multiple imputation
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HV	Hydrogel Vehicle Insert
ICH	International Council on Harmonization
ICF	Informed Consent Form
ID	Subject Identification
IEC	Independent Ethics Committee
IOP	Intraocular Pressure
IP	Investigational Product
IRB	Institutional Review Board
IRT	Interactive Response Technology
ITT	Intent-to-Treat

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Abbreviation	**Definition**
IUD	Intra-Uterine Device
KCS	Keratoconjunctivitis Sicca
LogMAR	Logarithm of the Minimum Angle of Resolution
NDA	New Drug Application
NEI	National Eye Institute
NSAID	Nonsteroidal Anti-Inflammatory Drug
PP	Per Protocol
OD	Right Eye
OS	Left eye
OSDI	Ocular Surface Disease Index
OTX	Ocular Therapeutix
OTX-DED	Dexamethasone Ophthalmic Insert
PK	Pharmacokinetics
QD	quaque die (one a day)
SAE	Serious Adverse Event/Experience
SUSAR	Suspected Unexpected Serious Adverse Reactions
TBUT	Tear Film Break Up Time
tCFS	Total Corneal Fluorescein Staining
UPT	Urine Pregnancy Test
US	United States
VA	Visual Acuity
VAS	Visual Analog Scale
WOCBP	Women of Childbearing Potential

1. INTRODUCTION AND OBJECTIVES OF ANALYSIS

1.1. Introduction

Dry eye disease (DED) is a multifactorial disorder of the tears and ocular surface characterized by symptoms of dryness and irritation. Although the pathogenesis of dry eye disease is not fully understood, it is recognized that inflammation has a prominent role in the development and propagation of this debilitating condition. Factors that adversely affect tear film stability and osmolarity can induce ocular surface damage and initiate an inflammatory cascade that generates innate and adaptive immune responses. These immunoinflammatory responses lead to further ocular surface damage and the development of a self-perpetuating inflammatory cycle.

DED flares are rapid-onset, inflammation driven responses to a variety of triggers that typically cannot be adequately managed with patients ongoing maintenance therapy – such as artificial tears and chronic prescribed therapies (ASCRS, 2019). Ocular surface inflammation plays a key role in all types of DED and conjunctival hyperemia in DED is a good indicator of inflammation (Baudouin, 2018). Acute DED flares are driven by both innate and adaptive immune responses (Perez et al., 2020) and corticosteroids targets both innate and adaptive immune response (Jones et al., 2017).

Ocular Therapeutix has designed a resorbable intracanalicular insert as the platform for drug delivery products which can be used to deliver various active pharmaceutical ingredients approved by the Food and Drug Administration (FDA).

OTX-DED is an intracanalicular insert that consists of two main components: dexamethasone and polyethylene glycol (PEG) based hydrogel conjugated with fluorescein. OTX-DED contains approximately 0.2 mg or 0.3 mg of dexamethasone and is designed to provide a sustained and tapered release of dexamethasone for up to 14 and 21 days, respectively. Over this time and through hydrolysis, OTX-DED softens, liquefies and is cleared through the nasolacrimal duct without the need for removal.

2. STUDY DESIGN

2.1. Synopsis of Study Design

This is a randomized, multi-center, double-masked, vehicle-controlled, phase 2 clinical study designed to evaluate the efficacy and safety of OTX-DED (dexamethasone ophthalmic insert) for intracanalicular use for the short-term treatment of DED. Approximately 150 subjects (300 eyes) will be enrolled in this study at approximately 15 sites in the US. Subjects will be a randomized to one of three treatment groups as noted in Table 2. Both eyes will be treated with the same treatment.

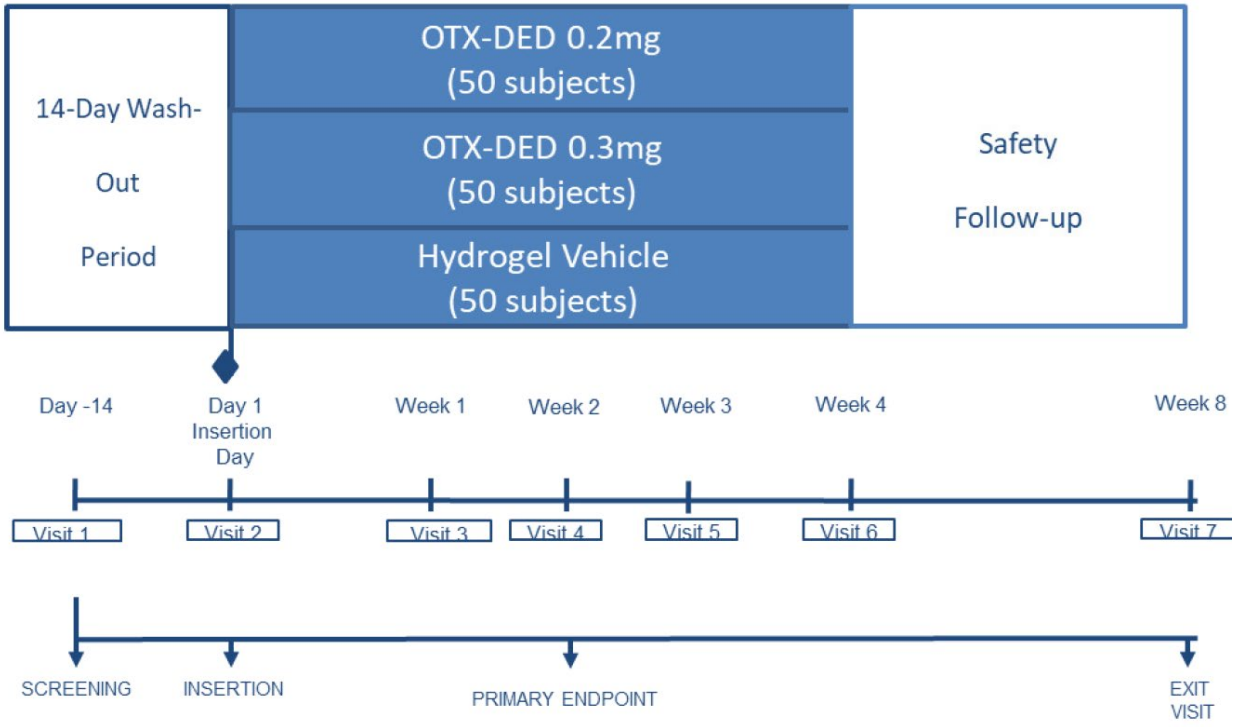
Table 1 Treatment Assignment Paradigm

Name Number of Subjects	Number of Subjects
OTX-DED 0.2mg Dexamethasone Ophthalmic Insert	50
OTX-DED 0.3mg Dexamethasone Ophthalmic Insert	50
Hydrogel Vehicle (HV) Insert 50	50

Subjects will undergo Screening 14 days prior to Insertion/Day 1 (Visit 2). At Visit 2 (Insertion/Day 1) eligibility will be confirmed and subjects who are eligible will be randomly assigned to one of three treatment groups. The treatment follow-up visits will occur at Visit 3 (Day 8), Visit 4 (Day 15), Visit 5 (Day 22), Visit 6 (Day 29), Visit 7 (Day 57).

This study will be conducted per the schedule shown in Figure 1

Figure 1: Study schematic



2.2. Objectives of Statistical Analysis

To assess the efficacy and safety of OTX-DED for the short-term treatment of signs and symptoms of Dry Eye Disease.

2.3. Randomization and Masking

Subjects will be randomized to treatment assignment.

A randomization schedule will be computer-generated by a qualified biostatistician independent of the study conduct or project team and uploaded into the EDC system. The EDC/IRT system will be used for randomization and unmasking. The OTX-DED and HV inserts administered to subjects at randomization in the double-masked treatment phase will be comparable in appearance. Eligible subjects will be randomly assigned to one of three treatment groups (OTX-DED 0.2mg, OTX-DED 0.3mg, or HV) in a 1:1:1 ratio.

Study subjects and investigators and their staff will be masked to the identity of treatment until the final database is locked. The Sponsor's personnel involved with the conduct and monitoring of the study will remain masked until completion of the study and database lock.

2.4. Stopping Rules and Unmasking

Should it become apparent during the trial that there is a significant safety concern or there is an issue with enrollment, the trial may be terminated. In addition, should information become known during the course of the trial that would negatively impact the trial, the trial may be terminated. In addition, the FDA or another regulatory authority may terminate the trial.

Appropriate precautions must be taken to prevent unauthorized access to the randomization scheme. Unless the subject's safety requires otherwise and if time permits, the decision to unmask a treatment assignment is to be made jointly by the Investigator and Sponsor's medical monitor.

If unmasking is required, the integrity of the study assessments and objectives will be maintained by limiting access to the unmasked data.

2.5. Study Procedures

The schedule of assessments, as outlined in the study protocol, is provided in Table 1.

Table 2 Schedule of Assessments

Study Parameter	Screening/ Baseline Visit Day -14 (-16 to -12)	Insertion Day 1	Follow-up Day 8 Week 1 ±2 days	Follow-up Day 15 Week 2 ±2 days	Follow-up Day 22 Week 3 ±2 days	Follow-up Day 29 Week 4[6] ±3 days	Follow-Up Day 57 Week 8 (± 10 days)
Visit	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Informed Consent	X						
Determine Eligibility	X	X					
Demographic Information	X						
Medical/Ophthalmic History	X						
Record Medications	X	X	X	X	X	X	X
Record Adverse Events[3,7]		X	X	X	X	X	X
Urine Pregnancy Test[1]		X					X
VAS for Eye Dryness	X	X	X	X	X	X	X
OSDI	X	X	X	X	X	X	X
SPEED	X	X	X	X	X	X	X
Conjunctival Hyperemia photography		X		X			
Assessment of BCVA	X	X	X	X	X	X	X
Slit Lamp Biomicroscopy (including punctum assessment)	X	X	X	X	X	X	X
Investigator Rated Conjunctival Hyperemia grade	X	X	X	X	X	X	X
Corneal Fluorescein Staining Using the NEI Scale	X	X	X	X	X	X	X
OTX-DED or HV Insert Presence by Visual Assessment			X	X	X	X	X
Ease of Visualization as assessed by Investigator			X	X	X	X	X
Unanesthetized Schirmer Tear Test	X						
Tear Film PK performed using Schirmer Strips[3,4,5]		X	X	X	X	X	X
IOP Measurement	X	X	X	X	X	X	X
Fundus Exam[2]	X						X
Punctum Size Assessment		X					X
Randomization		X					
OTX-DED or HV Insert Placement[3]		X					
Ease of Insertion as assessed by the Investigator[3]		X					
Dispense Sponsor Supplied Artificial Tears (if needed) and Daily Diary	X	X	X	X	X	X	
Collect/review Daily Subject Diary for Artificial Tear Use (as needed)		X	X	X	X	X	X

1 A negative UPT is required for women of childbearing age to be included in the study.
2 Dilated fundus examination at screening; undilated fundus examination at Week 8.
3 Prior to insertion, a clinical determination of the absence of infection will be made based on the lack of erythema or discharge at the punctum. If placement is only successful in one eye, subjects should be asked to return within 1-3 days to attempt insertion in the other eye. The following items should be repeated at the return visit, Insert Placement, Ease of Insertion, Tear Film PK and AEs.
4 Tear Film PK will be collected at 1 hour (±30 minutes), 2 hours (±30 minutes), and 4 hours (±30 minutes) post insertion on Day 1. Tear Film PK should be collected using Schirmer strips.
5 Tear Film PK will be collected using Schirmer Tests strips. These will be collected and sent to the central lab for analysis. See lab manual.
6 Early termination subjects should complete assessments based on the final visit (Visit 7) schedule of assessments.
7 Signs, symptoms, conditions occurring prior to insertion on Day 1 should be captured as medical history.

2.6. Efficacy, Pharmacokinetic, and Safety Variables

2.6.1. Efficacy Variables

2.6.1.1. Primary Efficacy Variables

1- Photographic assessment of bulbar conjunctival hyperemia (evaluated via central reading center) change from baseline (CFB) at Visit 4 (Day 15) in study eye.

The primary analysis will be based on the change from baseline in worst zone of photographic assessment of bulbar conjunctival hyperemia at baseline in the study eye. The worst (highest grading score) zone will be determined among Nasal, Temporal, and Frontal zones of photographic assessment of bulbar conjunctival hyperemia; if there are equal scores in two or more zones, the worst zone will be selected in a preferential order with Nasal zone being the most preferred, followed by Temporal zone, and then by Frontal zone in study eye at baseline. Change from baseline in this same chosen zone at Visit 4 (Day 15) will be investigated.

2.6.1.2. Secondary Efficacy Variables

1- Severity of Eye Dryness Score (visual analogue scale [VAS]) and their CFB at Visit4 (Day 15) and other post-baseline visits.

2- Frequency Eye Dryness Score (visual analogue scale [VAS]) and their CFB at Visit4 (Day 15) and other post-baseline visits.

3- Investigator assessment of bulbar conjunctival hyperemia and their CFB at Visit 4 (Day 15) and other post-baseline visits,

4- Absolute value of photographic assessment of bulbar conjunctival hyperemia (evaluated via central reading center) at Visit 4 (Day 15).

For this analysis, similar to primary efficacy variable, the worst (highest grading score) zone will be determined among Nasal, Temporal, and Frontal zones of bulbar conjunctival hyperemia assessed by investigator; if equal scores in two or more zones, the worst zone will be selected in a preferential order with Nasal zone being the most preferred, followed by Temporal zone, and then by Frontal zone in study eye at baseline. Change from baseline in this same chosen zone at each post-baseline visit will be investigated.

5- Total Corneal Fluorescein Staining (tCFS) using National Eye Institute (NEI) scale and its CFB to each post-baseline visit.

6- CFS sub-regions using NEI scale and its CFB to each post-baseline visit.

7- Ocular Surface Disease Index questionnaire (OSDI©) questionnaire, CFB and absolute values at each post-baseline visit (total score, each of the three domains, and individual questions).

- OSDI total score will be calculated using below OSDI© formula:

$$Overall\ OSDI\ score\ = \frac{(Sum\ of\ scores\ for\ all\ questions\ answered)\ \times\ 25}{number\ of\ questions\ answered}$$

- OSDI score for each section (ABC) will be calculated using below formula:

$$OSDI\ score\ for\ each\ section\ (A, B, C) = \frac{(Sum\ of\ scores\ for\ all\ questions\ answered\ for\ the\ section)\ \times\ 25}{number\ of\ questions\ answered\ in\ that\ section}$$

The OSDI is scored on a scale of 0 to 100, with higher scores representing greater.

8- SPEED questionnaire (overall score and individual questions), CFB at each post-baseline visit.

2.6.1.3. Exploratory Efficacy Variables

1- Presence of OTX-DED or HV insert at all post-baseline visits

2- Ease of insertion as assessed by the Investigator

3- Ease of visualization as assessed by the Investigator

4- Schirmer Test without anesthesia CFB

2.6.2. Safety Variables

1- Ocular and Non-Ocular Adverse Events

2- Best Corrected Visual Acuity (BCVA): LogMAR Visual Acuity (VA) score at each visit and its CFB at each post-baseline visit.

3- Slit Lamp biomicroscopy parameters by location (Eyelid Vascularity (vascular engorgement), Meibomian Glands (upper and lower lid orifice plugging), Punctal Appearance, Lid Apposition, Lashes, Cornea, Sclera, Conjunctiva, Anterior Chamber, Iris, Pupil, Lens) at baseline and at each post-baseline visit.

4- Intraocular Pressure (IOP) measurement (mmHg) and CFB by visit.

5- Fundus Examination: Vitreous, Retina, Macula, Choroid, Optic Nerve at baseline and at Visit 7 (Day 57).

6- Artificial tear use during the study: the use of Artificial tears since last visit at each post-baseline visit.

3. SUBJECT POPULATIONS

3.1. Population Definitions

1. **Intent-to-Treat (ITT):** The ITT population will include all randomized subjects. Subjects who are randomized but not treated due to procedural screen failures will be included as part of ITT population.
2. **Modified Intent-to-Treat (mITT)**: The Modified ITT will include all subjects in ITT who received the insert in the study eye, investigational product (IP) (OTX-DED or HV). The mITT population will be used as the primary efficacy analysis population and will also be used for all efficacy endpoints. Analyses performed on the mITT population will be according to the treatment the subject are randomized.
3. **Per Protocol (PP):** The PP population will include all mITT subjects who do not have any major protocol deviation which is likely to seriously affect the safety and efficacy outcomes of the study and who do not report excessive artificial tear use defined as greater or equal to 30 drops between Insertion/Day 1 (Visit 2) and Week 2 (Visit 4). Analysis on the PP population will be used as secondary efficacy analysis and will be performed for select efficacy endpoints, analyzing subjects under the treatment actually received. Important protocol deviations will be identified prior to locking the study database.
4. **Safety**: The Safety population will include all randomized subjects who received Investigational Product (IP) (OTX-DED or HV). Analyses performed on the Safety population will be according to the treatment the subject actually received.

3.2. Protocol Deviations

At the discretion of the sponsor, major protocol deviations as determined by a review of the data prior to unmasking of the study results and the conduct of statistical analyses may result in the removal of a subject's data from the PP Population. This file will include a description of the protocol deviations, as well as classification of major or minor. It will be finalized prior to hard database lock and unmasking. Major protocol deviations will be summarized by treatment groups and overall. The data listing for all protocol violations will be also presented.

4. STATISTICAL METHODS

4.1. Sample Size Justification

This study is not powered to show statistical significance but will provide initial estimates and trends of the endpoints for use in future trial designs. The sample size will allow for safety information to be obtained, while still limiting the number of subjects exposed to the IP. Statistical analyses will be descriptive.

The trial will include approximately 150 subjects (300 eyes) from approximately 15 sites.

4.2. General Statistical Methods and Data Handling

4.2.1. General Methods

All output will be incorporated into Microsoft Excel or Word files, sorted, and labeled according to the International Council on Harmonisation (ICH) recommendations, and formatted to the appropriate page size(s).

Tabulations will be produced for appropriate demographic, baseline, efficacy, and safety parameters. For categorical variables, summary tabulations of the number and percentage of each category of the parameter will be presented by treatment groups and visits (as applicable). For continuous variables, the n, mean, standard deviation, median, minimum, and maximum values will be presented.

The baseline visit will be defined as the last non-missing measure prior to initiation of IP. Differences between treatment groups will be calculated as OTX-DED minus HV and change from baseline will be calculated as follow up visit minus baseline visit values. In addition to looking at the individual formulations, the two formulations of OTX-DED will be combined and summarized.

All statistical testing will be done at the two-sided alpha level of 0.05, with no adjustments due to multiple endpoints testing.

4.2.2. Unit of Analysis

Both eyes will be treated with the same treatment/formulation. The study eye and non-study eye will be determined at the time of programing as follows. If both eyes qualify, the eye having the highest (worst) grading score among Nasal, Temporal, and Frontal zones from the photographic assessment of conjunctival hyperemia at baseline will be designated as the study eye and the other eye will be designated as the non-study eye. If both eyes have the same photographic assessment conjunctival hyperemia the highest (worst) grade score, the right eye will be the study eye. If only one eye qualifies, that eye will be the study eye, but both eyes will still receive the same treatment/formulation and be included in the safety analysis. If only an insert is successfully placed in one eye, then that eye will be the study eye, if it qualifies for the study.

The unit of analysis will be the study eye (primary study eye) with the worst zone chosen for bulbar conjunctival hyperemia summaries and analyses. If there are equal scores in two or more zones, the worst zone will be selected in a preferential order with Nasal zone being the most preferred, followed by Temporal zone, and then by Frontal zone. Efficacy summaries will be presented for both study and non-study eyes. Safety summaries will include summaries of both study and non-study eyes. All primary, sensitivity, secondary, and exploratory endpoints will be analyzed for both study and non-study eyes, separately. All summaries will be presented by treatment groups and visits, where appropriate. In addition to looking at the individual formulations, the two formulations of OTX-DED will be combined and summarized.

4.2.3. Computing Environment

All descriptive statistical analyses will be performed using SAS statistical software (Version 9.4), unless otherwise noted. Medical History and adverse events will be coding using Medical Dictionary for Regulatory Activities (MedDRA) version 24.0 - Mar 2021. Concomitant medications will be coded using World Health Organization (WHO) DD B3 WHO Drug DDE – Sep 2020.

4.2.4. Methods of Pooling Data

The two OTX-DED formulations will be presented separately as individual formulations and combined.

4.2.5. Adjustments for Covariates

The baseline value will be included in the analysis of covariance (ANCOVA) model as a covariate for adjustment.

4.2.6. Multiple Comparisons/Multiplicity

To control the type I error for the hypothesis testing of the primary endpoint, least square means will be used to make treatment comparisons using Dunnett's adjustment for multiple comparisons to a control.

As this is a Phase 2 study, no adjustments to alpha will be made for testing of multiple endpoints.

4.2.7. Subpopulations

No analyses of subgroups of subjects are planned.

4.2.8. Withdrawals, Dropouts, Loss to Follow-up

Any subject who wishes to voluntarily discontinue study drug or withdraw from participation in the study for any reason is entitled to do so without obligation. IP may be discontinued (insert removed) and any subject may be discontinued from study participation at any time during the study at the discretion of the Investigator or the Sponsor for any reason.

In the event that study discontinuation of a randomized subject is necessary, the Investigator should make every attempt to have the subject complete Visit 7 assessments as soon as possible. The reason for premature discontinuation should be recorded in the subject chart and entered in the eCRF.

Subjects who withdraw will not be replaced.

4.2.9. Missing, Unused, and Spurious Data

If the start date of an AE is partially or completely missing, the date will be compared as far as possible with the date of the start of administration of study drug. The AE will be assumed to be treatment-emergent if it cannot be definitively shown that the AE did not occur or worsen during the treatment-emergent period (worst case approach). The imputed dates will only be used to classify events as treatment emergent and will only be used in the table analyses. Listings will display the available date data.

The following general rules will be used:

- If the start day is missing but the start month and year are complete, an AE will only be excluded as being treatment-emergent if the start month/year is before the month/year

of study drug administration or if the date of resolution is before study drug administration.

- If the start day and month are missing but the start year is complete, an AE will only be excluded as being treatment-emergent if start year is before the year of study drug administration or if the date of resolution is before study drug administration.
- If the start date is completely missing, an AE will be considered treatment-emergent unless the date of resolution is before study drug administration.

4.2.9.1. Imputation Method for Primary Efficacy Endpoints

To impute missing, Markov Chain Monte Carlo (MCMC) multiple imputation (MI), Fully Conditional Specification multiple imputation (FCS MI) and LOCF methods will be used.

For MI, we will have below phases:

1. Imputation Phase: in this phase, the missing data will be imputed using SAS PROC MI and a complete data set will be created. This process of imputation will be repeated 25 times. Therefore, 25 compete datasets will be created. In this phase, treatment group, baseline value, and post-baseline visits will be included in multiple imputation model.

2. Analysis Phase: each of the 25 complete data sets will be analyzed independently using desired statistical method (e.g. ANCOVA for primary efficacy analysis).

3. Pooling Phase: in this phase the estimates obtained from each analyzed complete dataset from phase 2, will be combined using PROC MIANALYZE.

The primary analyses of efficacy data will use MCMC MI methodology to impute missing data. The expectation and maximization (EM) algorithm will be used to estimate the mean vector and the covariance assuming a missing at random (MAR) distribution. The MAR distribution assumes the probability that a value is missing does not depend on the true values of the missing items. The imputation phase will create 25 copies of the dataset which will contain different estimates of the missing values. The 25 complete datasets will then be analyzed to produce several sets of parameter estimates and standard errors, which will then be pooled to create a single set of results.

Last observation carried forward (LOCF) method and Fully Conditional Specification multiple imputation (FCS MI) with 200 burn-in iterations and 25 times of imputation under the assumption of missingness at random will be used for sensitivity analyses (Section 4.7.2).

4.2.10. Visit Windows

Visit windows will be calculated based on the Schedule of Assessments in Table 1. Any visits or procedures performed outside the scheduled visits will be documented in the Unscheduled Visit page of the eCRF. Although there is a visit window around the expected visit date, nominal visits will be used for the per-visit analyses.

4.3. Interim Analyses

An interim analysis was not planned for this study.

4.4. Subject Disposition

A summary of subject disposition will be tabulated, including the number screened, screened failure, and procedural screen failures will be presented. Subjects are considered procedural screen failures if the investigator is unsuccessful at placing the OTXDED or HV intracanalicular ophthalmic insert in both eyes (i.e. neither eye has an insert). Procedural screen failures will be exited from the study at that time, and will be included in the disposition tables as 'randomized, not treated'. Subjects will be followed per protocol if the investigator successfully places one insert.

The number and percentage of subjects included in each of the analysis populations (ITT, mITT, PP, Safety) will be presented by treatment groups and overall. Subject disposition events including randomization, treated, completed study, withdrawal prior to completing the study, and reasons for withdrawal will be summarized by treatment groups and overall.

A by-subject listing of study completion information, including the reason for premature study withdrawal, if applicable, will be presented.

4.5. Demographic and Baseline Characteristics

Baseline and demographic information will be summarized for the mITT population using descriptive statistics. Demographic characteristic information will include Age (year), Age categories (<65, ≥65), Gender (Male, Female), Ethnicity (Hispanic or Latino, Not Hispanic or Latino), and Race (White, Black or African American, Asian, American Indian or Alaska Native, Native Hawaiian or Other Pacific Islander, Unknown, Other).

Demographic and Baseline data will be provided in data listings.

4.5.1. Baseline Ocular Assessments

The following baseline assessments will be presented using descriptive statistics for mITT population by treatment group and overall.

The assessments below will be assessed:

1. Bulbar conjunctival hyperemia (worst zone, Nasal, Temporal, Frontal, Total of 3 zones)
2. Eye Dryness Severity (VAS) Score
3. Eye Dryness Frequency (VAS) Score
4. Investigator assessment of bulbar conjunctival hyperemia
5. Corneal Fluorescein Staining (tCFS) by study eye
6. CFS sub-regions using NEI scale by study eye
7. OSDI total score (0-100) and the score of its three domains
8. SPEED Total Score (0-28)
9. Best Corrected Visual Acuity (BCVA) LogMAR Score
10. IOP Measurement (mmHg)
11. Unanesthetized Schirmer's Test

4.5.2. Ocular and Non-Ocular Medical History

Medical history, including ocular medical history, will be coded using MedDRA Version 24.0 – Mar 2021. Non-ocular medical history will be summarized in the mITT population by system organ class (SOC) and preferred term (PT) by treatment group and overall. SOC will be sorted alphabetically. PT will be sorted by descending frequency overall within each SOC. Subjects with a particular medical history event or medical history class will be counted once at the PT level and once at the SOC level. Ocular medical history will be summarized at the eye and subject levels by treatment group and overall, for the mITT population with separate summaries for the study eye and non-study eye.

Data listings will be provided for medical history as well.

4.6. Treatment Exposure

Details of study drug administration, including duration of treatment will be tabulated and presented for the Safety population. The duration of exposure will be calculated in days as latest date investigator confirms insert is not present in either eye minus date of the earliest insertion in either eye plus 1 and summarized using descriptive statistics by treatment group and

overall. Treatment exposure for subjects who discontinue the study prior to the insert no longer being present in both eyes, will be calculated in days as date of last insert visualization in either eye minus date of the earliest insertion in either eye plus 1. In addition, treatment exposure for subjects who discontinue the study prior to the insert no longer being present in both eyes will be set to the longest observed insert exposure as an additional analysis.

All dosing information will be presented in a data listing.

4.7. Efficacy Evaluation

The efficacy summaries and analyses will be presented for both study and non-study eyes.

The Primary efficacy analysis of the bulbar conjunctival hyperemia will be conducted using the mITT and PP populations.

4.7.1. Primary Analyses

For the primary efficacy endpoint analyses of change from baseline in photographic assessment of bulbar conjunctival hyperemia (evaluated by central reading center) worst zone in the study eye at Visit4 (Day 15) (as defined in section 2.6.1.1), the following modeling and analyses will be performed on mITT population, observed data:

An analysis of covariance (ANCOVA) model will be run to estimate least square (LS) means treatment. This model will include the baseline value as a covariate for adjustment and treatment group as the sole factor. Least square means will be used to make treatment comparisons using Dunnett's adjustment for multiple comparisons to a control. Statistical significance of treatment differences will be determined using a two-sided significance level of $\alpha = 0.05$.

In the ANCOVA model, LS means treatment group comparison of OTX-DED 0.2 mg to HV, OTX-DED 0.3 mg to HV, and Overall OTX-DED (combined OTX-DED 0.2 mg and OTX-DED 0.3 mg using sample size weights of OTX-DED 0.2 mg and OTX-DED 0.3 mg groups as coefficients for the parameters) to HV will be reported. Unadjusted treatment group analyses using two-sample t-test and Wilcoxon rank sum test will be run on these treatment group combinations.

Each bulbar conjunctival hyperemia zone (Nasal, Temporal, and Frontal) and the Total of the three zones in study eye will be analyzed in a manner similar to the primary efficacy variable as described above.

The same analyses will be done for the non-study eye. For non-study eye, the worst zone similar to worst zone in study eye (details in section 2.6.1.1) will be independently determined using photographic assessment bulbar conjunctival hyperemia in non-study eye at baseline.

The primary efficacy endpoints will also be analyzed on PP population.

4.7.2. Sensitivity Analyses

To determine robustness of results, the sensitivity analyses will be performed for the primary efficacy endpoint, bulbar conjunctival hyperemia at Visit4 (Day 15), on the mITT and PP populations by using:

1. MCMC MI
2. last observation carried forward (LOCF)
3. FCS MI.

As sensitivity analyses, LOCF will be analyzed in a manner similar to the primary efficacy variable (details in section 4.7.1). MCMC MI and FCS MI will be analyzed in a manner similar to the primary efficacy variable (details in section 4.7.1), however, for treatment comparisons, least square means will not be adjusted for multiple comparison and for the unadjusted treatment group comparisons, only t-test will be used.

4.7.3. Secondary Analyses

The below secondary efficacy endpoints will be analyzed in a manner similar to the primary efficacy variable using ANCOVA model of change from baseline using observed data:

1. Severity of eye dryness score at Visit 4 (Day 15) and other post-baseline visits,
2. Frequency of eye dryness score at Visit 4 (Day 15) and other post-baseline visits,
3. Investigator assessment of bulbar conjunctival hyperemia in worst zone, Nasal, Temporal, Frontal zones, and Total at each post-baseline visit,
4. Total corneal fluorescein staining (tCFS) and CFS sub-regions at each post-baseline visit.

In addition, sensitivity analyses done for the primary efficacy endpoint (section 4.7.2) will also be performed on severity and frequency of eye dryness scores, investigator assessment of bulbar conjunctival hyperemia, tCFS, and CFS sub-regions endpoints.

The below secondary efficacy endpoints will be analyzed using ANCOVA model of change from baseline at each post-baseline visit similar to primary efficacy endpoint analysis (section 4.7.1) using observed data:

5. Ocular Surface Disease Index questionnaire (OSDI©) at each post-baseline visit (total score, each of the three domains, and individual questions) and
6. SPEED questionnaire (overall score and individual questions), CFB at each post-baseline visit will be analyzed

All the secondary endpoints will be analyzed on mITT and PP populations; therefore, related summary tables will be reported for both mITT and PP populations.

4.7.4. Exploratory Analyses

Ease of insertion as assessed by the Investigator at Visit 2 or Visit 2B, presence of OTX-DED or HV insert and ease of visualization as assessed by the Investigator at all post-baseline visits will be summarized using discrete statistics (number and percentage) and will be tested between treatment groups (OTX-DED 0.2 mg to HV, OTX-DED 0.3 mg to HV, and Overall OTX-DED (combined OTX-DED 0.2 mg and OTX-DED 0.3 mg) to HV). To analyze ease of insertion as assessed by the Investigator and ease of visualization as assessed by the Investigator, the Pearson Chi-squared statistic will be used. To analyze presence of insert, Fisher's exact Test will be used, and Clopper-Pearson method will be used to calculate 95% confidence interval of the proportion of subject with presence of insert; to calculate 95% confidence interval of the proportion difference, the Chan and Zhang (1999) method will be used.

The Schirmer Test without anesthesia will be summarized using descriptive statistics by treatment group and overall and it will be analyzed using ANCOVA model of both change from baseline and absolute value at each post-baseline visit similar to primary efficacy endpoint analysis (section 4.7.1) using observed data.

The exploratory analyses will be done on mITT populations

4.8. Pharmacokinetic Evaluations

PK analysis will be described separately.

4.9. Safety Analyses

All safety analyses will be conducted on the Safety population.

4.9.1. Adverse Events

Adverse events will be coded using MedDRA Version 24.0 – Mar 2021 and displayed in tables and listings using System Organ Class (SOC) and Preferred Term (PT).

Analyses of adverse events will be performed for those events that are considered treatment emergent adverse events (TEAE), where treatment emergent is defined as any adverse event

with onset on or after the first dose of study medication through the end of the study or any event that was present at baseline but worsened in intensity or was considered drug-related by the investigator through the end of the study.

The safety will be assessed by incidence of TEAEs, therefore, in any tabulation, a subject contributes only once to the count for a given adverse event (SOC or preferred term). An overall summary of TEAEs will be presented including the number of events and the number of the subjects with events along with percentages by treatment group and overall. The overall summary will include subjects with AE leading to death, subjects with TEAEs leading to insert removal and subjects with TEAEs leading to subject withdrawal. In summary tables, SOC will be presented alphabetically and events within SOC will be presented by decreasing frequency count based on the total column.

The frequency and percentage of subjects with any TEAE, any serious TEAE, any TEAEs by relationship to treatment (unrelated, related), any serious TEAEs by relationship to treatment (unrelated, related), any TEAE by severity (mild, moderate, or severe) will be summarized separately for ocular and non-ocular events by treatment group and overall. Separate summaries for ocular TEAEs and non-ocular TEAEs by preferred terms in decreasing frequency based on all subjects will be tabulated.

All adverse events will be listed in subject data listings.

By-subject listings also will be provided for the following: subject deaths; serious adverse events; and adverse events leading to withdrawal.

4.9.2. Non-AE Safety Analyses

The following safety assessments will be summarized using descriptive statistics for Safety population by treatment group and overall. Assessments will be presented for study eye and non-study eye separately.

1. Best Corrected Visual Acuity (BCVA)

   LogMAR Visual Acuity (VA) score for each visit, its CFB, and loss 3 Lines or more in BCVA LogMAR Score from Baseline at each post-baseline visit will be calculated and summarized using descriptive statistics.

2. Slit-Lamp biomicroscopy

   The evaluation of normal, mild, moderate, severe, and very severe for each eye in Eyelid Vascularity (vascular engorgement) and Meibomian Glands (upper and lower lid orifice plugging) , as well as normal, abnormal non-clinically significant, and abnormal clinically significant for each eye in Punctal Appearance, Lid Apposition, Lashes, Conjunctiva,

Sclera, Cornea, Anterior Chamber, Iris, Pupil, and Lens will be tabulated for each visit by using the frequency and percentage.

3. Intraocular Pressure (IOP) measurement (mmHg)

Absolute values and CFB of IOP at each post-baseline visit, absolute IOP measurement ≥ 30 mmHg at each visit, and CFB in IOP > 5 mmHg as well as >10 mmHg at each post-baseline visit will be summarized using descriptive statistics.

4. Fundus Examination

The evaluation of normal, abnormal non-clinically significant, and abnormal clinically significant for each eye in vitreous, retina, macula, choroid, and optic nerve exam will be tabulated for visit 1 and visit 7 by using the frequency and percentage. The absolute cup-to-disc ratio at baseline and Visit 7 (Day 57) and its CFB at Visit 7 (Day 57) will be presented using descriptive statistics.

5. Artificial tears use during the study

The use of artificial tears, based on the Daily Subject Diary, since last visit will be tabulated for each post-baseline visit by using frequency and percentage. Number of times AT used since last visit will be reported among all subjects who have any daily subject dairy data since the last visit using descriptive statistics.

4.9.3. Laboratory Data

Safety laboratory tests will not be collected. Urine pregnancy test results will be captured at Visit 2 (Day 1) and Visit 7 (Day 57). Urine pregnancy test results will be provided in data listing.

4.9.4. Vital Signs and Physical Examinations

Vital signs and physical examinations data will not be collected.

4.9.5. Concomitant Medications

Concomitant medications will be coded using the WHO Drug dictionary, DD B3 WHO Drug DDE – Sep 2020. Results will be tabulated by Anatomic Therapeutic Class (ATC) and preferred term.

Concomitant medications will be defined as those medications that were initiated after study drug administration or those that were ongoing at the time of study drug administration. If the start date or end date of a medication is partially missing, the date will be compared as far as possible with the date of the start of administration of study drug. The medication will be

assumed to be prior medication if it cannot be definitively shown that the medication did not start or continue during the treatment period.

Non-ocular prior and concomitant medications will be summarized by higher ATC level 3 (therapeutic subgroup) and preferred term by treatment group and overall, in the Safety population. ATC Level 3 terms will be sorted alphabetically. Preferred terms will be sorted by descending frequency overall within ATC Level 3 term. Subjects receiving a particular medication or medication of ATC Level 3 will be counted once at the preferred term level and once at ATC Level 3.

Ocular prior and concomitant medications will be summarized at the eye level, separately for study and non-study eyes, and at the subject level (both eyes) by ATC Level 3 and preferred term by treatment group and overall, in the Safety population.

All prior and concomitant medications information will be presented in the data listing.

5. CHANGES TO PLANNED ANALYSES

The primary, sensitivity, and secondary efficacy endpoints will be analyzed using both mITT and PP populations.

Protocol pre-specified primary analysis is multiple imputation using MCMC with Dunnett's adjustment. Upon further technical research, multiple imputation using MCMC with Dunnett's adjustment can't be correctly implemented. Due to the current limitation of multiple imputation with multiplicity correction, the primary analysis will be conducted using observed data with Dunnett's adjustment.

For sensitivity analyses, MCMC MI, LOCF, and FCS MI will be used. For analyses using MCMC MI and FCS MI, for treatment comparisons, least square means will not be adjusted for multiple comparison and for the unadjusted treatment group comparisons, only t-test will be used.

For severity and frequency eye dryness (VAS), investigator assessment of bulbar conjunctival hyperemia, and tCFS and its sub-regions, the main analysis will be based on observed data and sensitivity analyses will be done using MCMC MI, LOCF, and FCS MI.

For the presence of insert, the groups will be compared using observed data by Fisher's exact test. Clopper-Pearson method will be used to calculate 95% confidence interval of the proportion of subject with presence of insert; to calculate 95% confidence interval of the proportion difference, the Chan and Zhang (1999) method will be used.

6. REFERENCES

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7. CLINICAL STUDY REPORT APPENDICES

7.1. SAS Codes

7.1.1. SAS codes to impute the missing using MCMC MI

```
***** Bulbar Conjunctival Hyperemia multiple imputations *****;
***** Photographic Assessments *****;
PROC MI DATA=pr1t SEED=738493 OUT=pr1miyp MINIMUM=0 MAXIMUM=4 ROUND=1 NIMPUTE=25;
        MCMC INITIAL=EM;
        by PARAMN PARAMCD;
        where SEFL="Y" and PARAMCD in ("BCHSCTP" "BCHSCFP" "BCHSCNP");
       /* TRTPN_FG1 TRTPN_FG2 are dummy variables based on TRTPN*/
        VAR TRTPN_FG1 TRTPN_FG2 AVAL2 AVAL4;
RUN;
***** Investigator Assessments *****;
PROC MI DATA=pr1t SEED=738493 OUT=pr1miyi MINIMUM=0 MAXIMUM=4 ROUND=1 NIMPUTE=25;
        MCMC INITIAL=EM;
        by PARAMN PARAMCD;
        where SEFL="Y" and PARAMCD in ("BCHSCTI" "BCHSCFI" "BCHSCNI");
        VAR TRTPN_FG1 TRTPN_FG2 AVAL2 AVAL3 AVAL4 AVAL5 AVAL6 AVAL7;
RUN;
```

7.1.2. SAS codes to impute the missing using FCS MI

```
***** Bulbar Conjunctival Hyperemia multiple imputations *****;
***** Photographic Assessments *****;
PROC MI DATA=pr1t SEED=918411 OUT=pr1fcsyp MINIMUM=0 MAXIMUM=4 ROUND=1 NIMPUTE=25;
        by PARAMN PARAMCD;
        where SEFL="Y" and PARAMCD in ("BCHSCTP" "BCHSCFP" "BCHSCNP");
        class TRTPN;
        VAR TRTPN AVAL2 AVAL4;
        fcs nbiter=200 reg (AVAL2=TRTPN);
        fcs nbiter=200 reg (AVAL4=TRTPN AVAL2);
RUN;
***** Investigator Assessments *****;
PROC MI DATA=pr1t SEED=918411 OUT=pr1fcsyi MINIMUM=0 MAXIMUM=4 ROUND=1 NIMPUTE=25;
        by PARAMN PARAMCD;
        where SEFL="Y" and PARAMCD in ("BCHSCTI" "BCHSCFI" "BCHSCNI");
        class TRTPN;
        VAR TRTPN AVAL2 AVAL3 AVAL4 AVAL5 AVAL6 AVAL7;
        fcs nbiter=200 reg (AVAL2=TRTPN);
        fcs nbiter=200 reg (AVAL3=TRTPN AVAL2);
        fcs nbiter=200 reg (AVAL4=TRTPN AVAL2 AVAL3);
        fcs nbiter=200 reg (AVAL5=TRTPN AVAL2 AVAL3 AVAL4);
        fcs nbiter=200 reg (AVAL6=TRTPN AVAL2 AVAL3 AVAL4 AVAL5);
        fcs nbiter=200 reg (AVAL7=TRTPN AVAL2 AVAL3 AVAL4 AVAL5 AVAL6);
RUN;
```

7.1.3. SAS codes to run ANCOVA (using PROC MIXED) on MI data

```
*** MULTIPLE IMPUTATIONS ***;
*** PROC MIXED ***;
ods output LSMESTIMATEs=outlsmean0(rename=(label=PARAMETER));
proc mixed data=din;
        by AVISIT PARAMN IMPUT;
        CLASS TRTPN (ref=last);
        MODEL CHG=TRTPN BASE;
        LSMESTIMATE TRTPN   "OTX-DED 0.2MG" 1 0 0,
                "OTX-DED 0.3MG" 0 1 0,
               /* WG1 and WG2 are the sample size weighted coefficient for group 1 and 2 respectively*/
                "Overall OTX-DED" &WG1. &WG2. 0,
                "HV" 0 0 1,
                "OTX-DED 0.2MG vs HV" 1 0 -1 ,
                "OTX-DED 0.3MG vs HV" 0 1 -1,
                "Overall OTX-DED vs HV" &WG1. &WG2. -1 / cl;
run;

ods output ParameterEstimates=outlsmean1;
PROC MIANALYZE DATA=outlsmean0;
        modeleffects estimate;
        stderr stderr;
        by avisit PARAMN PARAMETER;
RUN;
```

7.1.4. SAS codes to run t-test on MI data

```
/* SAS code below for testing group 1 vs group 3*/
        ods output statistics=ttest1;
        proc ttest data=din;
                where TRTPN in (1 3)   ;
                by AVISIT PARAMN IMPUT;
                class TRTPN;
                var CHG;
        run;

        ods output parameterestimates=pdiff;
                proc mianalyze data=ttest2;
                modeleffects mean;
                stderr stderr;
                by AVISIT PARAMN Class;
        run;
```

7.1.5. SAS codes to run ANCOVA (using PROC MIXED) on Observed data and LOCF

```
*** PROC MIXED ***;
        ods output LSMESTIMATES=ls1(rename=(label=PARAMETER)) DIFFS=dun1;
                proc mixed data=din;
                by PARAMN AVISITN;
                CLASS TRTPN (ref=last);
                MODEL CHG=TRTPN BASE;
                lsmeans TRTPN / cl pdiff=control('3') adjust=Dunnett;
                LSMESTIMATE TRTPN  "OTX-DED 0.2MG" 1 0 0,
        "OTX-DED 0.3MG" 0 1 0,
        "Overall OTX-DED" &WG1. &WG2. 0,
        "HV" 0 0 1,
        "OTX-DED 0.2MG vs HV" 1 0 -1 ,
        "OTX-DED 0.3MG vs HV" 0 1 -1,
        "Overall OTX-DED vs HV" &WG1. &WG2. -1 / cl;
    run;
```

7.1.6. SAS codes to output 95% CI for proportions using Clopper-Pearson method and 95% CI for the proportion difference using Chan and Zhang (1999) method using observed and LOCF data

```
ods output RiskDiffCol1=freq1(where=(ROW in ("Row 1" "Row 2" "Difference")))
FishersExact=freq2(where=(NAME1="XP2_FISH"));
        proc freq data=din;
                where TRTPN in (1 3) and avisitn=4;
                table TRTPN*AVALN / norow nocol nopercent fisher;
                exact riskdiff(method=score);
        run;
```