

A Phase I/II Randomized Placebo-Controlled, Double-Blind, Single-Center, Tolerability And Preliminary Efficacy Clinical Trial Of Recombinant Human Deoxyribonuclease (Rhdnase) Eye Drops In Patients With Dry Eye Disease

Regulatory Sponsor: Sandeep Jain, MD
UIC Corneal Neurobiology Laboratory
L239, LIERI
1905 W Taylor Street
Chicago, IL 60612
Phone: 312-996-8936
Fax: 312-996-7770

Funding Sponsor: None

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SCHEDULE OF VISITS AND PROCEDURES

	Screening	Day 1 (Pre-Dose)	Day 1 (Post-Dose)	Week 2	Week 4	Week 6	Week 8	Week 10
Informed Consent	X							
Demographic Information	X							
Medical History/Con Meds	X							
Ophthalmic/Dry Eye History	X							
Vital Signs		X		X	X	X	X	X
Ophthalmic Examination (Slit lamp)	X	X		X	X	X	X	X
Ocular surface redness score	X	X		X	X	X	X	X
Schirmer 1 test and Rose Bengal staining	X						X	
Pregnancy Test (urine)*	X							
Best Spectacle Corrected Visual Acuity		X						X
Randomization visit		X						
First Study Medication		X						
Drug dispensation visits			X	X	X	X		
Instruction on study medication self-administration			X					
Ocular Surface Disease Index (OSDI)		X		X	X	X	X	X
Visual Analogue Scale (VAS)			X	X	X	X	X	
Physical inspection of nose & oropharynx				X	X	X	X	X
Clinical Global Impression (CGI)		X		X	X	X	X	X
Subject Global Assessment (SGI)		X		X	X	X	X	X
Adverse Events/ Concomitant Medications		X	X ⁺⁺	X	X	X	X	X

*If applicable ++ AEs only

ABBREVIATIONS

AE	Adverse Event
APC	Antigen-presenting cells
BSCVA	Best Spectacle Corrected Visual Acuity
EC	Ethics Committee
eDNA	Extracellular DNA
CNL	Corneal Neurobiology Laboratory
CRF	Case Report Form
CGI	Clinical Global Impression
DCF	Data Clarification Form
DED	Dry Eye Disease
DNase I	Deoxyribonuclease I
FDA	Food and Drug Administration
FVC	Forced vital capacity
GCP	Good Clinical Practice
ICH	International Conference on Harmonization
IB	Investigator's Brochure
IOP	Intraocular pressure
IRB	Institutional Review Board
KCS	Keratoconjunctivitis sicca
Net	Neutrophil extracellular trap
OSDI	Ocular Surface Disease Index
Otc	Over the counter
QID	Four times per day
rhDNase1	Recombinant human deoxyribonuclease I
SAE	Serious Adverse Event
SGL	Subject Global Assessment
SP	Substance P
UIC	University of Illinois at Chicago
VA	Visual Acuity
VAS	Visual Analog Scale

Study Summary

Title	A Phase I/II Randomized Placebo-Controlled, Double-Blind Single-Center, tolerability and preliminary efficacy clinical trial of recombinant human deoxyribonuclease (rhDNase) eye drops in patients with dry eye disease.
Short Title	DNase treatment for dry eyes
Protocol Number	UIC-CNBL-1001
Phase	Phase I/II
Methodology	Double-blind
Study Duration	10 weeks
Study Center(s)	Single-center
Objectives	To evaluate the tolerability and preliminary efficacy of rhDNase1 eye drops in patients with Dry Eye Disease.
Number of Subjects	72 (study drug at least 30, placebo at least 30)
Diagnosis and Main Inclusion Criteria	Men and women ≥ 18 years of age with a diagnosis of established Dry Eye Disease, with Schirmer I < 10 , Ocular surface staining ≥ 1 and OSDI mild (≥ 13).
Study Product, Dose, Route, Regimen	Study drug: rhDNase1 (Pulmozyme®), 0.1% eye drops four times a day for eight weeks. Control: drug vehicle eye drops four times a day for eight weeks.
Duration of administration	8 weeks
Reference therapy	None
Statistical Methodology	Descriptive statistics will be used for all primary and safety endpoints, when appropriate. A Wilcoxon test will be used to compare changes in subjective symptoms from Baseline to follow-up values at 2 weeks, 4 weeks, 6 weeks, 8 weeks and 10 weeks. Primary efficacy measure is the change in the OSDI reported between Baseline and Week 8. Primary safety measure is the proportion of patients at week 8 who were able to successfully complete a full eight weeks of therapy with topical administration four times per day (q.i.d).

1 Introduction

This document is a protocol for a human research study. This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

1.1 Background

Keratoconjunctivitis sicca (KCS) or Dry Eye Disease is a disease of the surface of the eye, tear film and related ocular tissues. Millions of people suffer from one form of the disease or another and its prevalence increases with age. Dry Eye Disease sufferers experience a broad range of symptoms including discomfort, irritation, burning, itching, redness, pain, gritty feeling, foreign body sensation, blurred vision and ocular fatigue. These symptoms can progress and lead to ulceration and perforation of the cornea, lead to increased ocular infections and even result in an inability to produce emotional tears.

Dry eye as a disease entity is considered to fall in to two broad categories: 1) Aqueous tear deficiency (as seen in Sjögren's syndrome) and 2) increased tear evaporation (as seen with infectious blepharitis, lid abnormalities, or associated with wearing contact lenses).

Until recently, the only treatment options for Dry Eye Disease (DED) were palliative in nature and limited to lubricating eyedrops or punctual occlusion procedures, which attempt to supplement natural tears, or improve the resistance time of the limited quantity of tears produced naturally by the patient.

Although DED pathogenesis is not fully understood, inflammation has a prominent role in DED symptom development and amplification.^{1,2} The current paradigm suggests that ocular surface inflammation is triggered by surface epithelium stress caused by tear hyperosmolarity. Inflammation is sustained by activated antigen-presenting cells (APCs) and T cells via the afferent and efferent limbs of the adaptive immune system.^{3,4}

The immunopathological events that sustain the systemic adaptive immune response in DED have been characterized.^{2,4} However, the mechanisms that activate the adaptive immune response are poorly understood. Ocular surface epithelial stress is a key initial event and a major source of innate cytokines and chemokines that can damage epithelial cells and activate APCs. Tear hyperosmolarity is recognized as an important stressor. However, tear replacement to decrease osmolarity provides limited therapeutic benefit. Therefore, additional stressors may activate DED ocular surface inflammation and link the innate and adaptive immune mechanisms.

The ocular surface epithelium undergoes continuous, dynamic turnover^{5,6}, which is increased in DED patients.⁷ Superficial corneal cells are shed into the precorneal tear film.^{8,9} The corneal epithelial cell shedding process, or desquamation, is regulated by apoptotic mechanisms.¹⁰ Dead and dying cells release extracellular DNA (eDNA), a type of damage-associated molecular pattern molecule, that can stimulate the innate immune system and link it to adaptive immune system.^{11,12} eDNA strands have been reported in corneal filaments, which are frequently present on the corneas of patients with severe DED.¹³ Desquamated cells in the precorneal tear film are a potential source of eDNA. Tear fluid nucleases, including lipocalin and DNase1, can hydrolyze and clear eDNA from the precorneal tear film.^{14,15} Additionally, tear fluid contains several neutrophil extracellular trap (NET) components. Neutrophils undergo a low level of recruitment on the ocular surface,¹⁶ and numerous neutrophils are

present in the tear film during ocular surface inflammation.¹⁷ Neutrophil elastase and histone proteins have also been reported in tear fluid.¹⁸ Taken together, these reports document the presence of eDNA, histones, neutrophils, neutrophil elastase, and nucleases in tear fluid and suggest mechanisms exist for the continual production and clearance of eDNA in the precorneal tear film.

We have performed investigations in dry eye disease patients that have yielded several important findings.¹⁹ First, eDNA and NETs are present in excessive amounts on the ocular surface of patients with severe, tear-deficient DED. Immunolocalization experiments revealed that the molecular components of NETs include histones, cathelicidin, and neutrophil elastase. Next, using a DNA digestion assay, we determined that tear fluid nuclease activity was >0.05 Kunitz units. Using a FRET-based assay, we found that tear fluid nuclease activity was reduced in DED patients. We determined that DNase I is a DNA hydrolyzing nuclease present in lacrimal glands and a normal concentration in tear fluid is 3.14 ng/ml, similar to that in serum and saliva. We also determined that mucoid films are present on the ocular surface or inferior conjunctival fornix in some patients with severe DED (particularly with GVHD) and are rich in neutrophils, eDNA, and NETs. Finally, exfoliated ocular surface cells from DED patients had increased expression of inflammatory cytokines and eDNA signaling pathway genes.

Taken together, these findings suggest that in healthy eyes, eDNA is produced in the precorneal tear film and cleared by tear fluid nucleases. In patients with severe DED, tear fluid nuclease deficiency allows eDNA, neutrophils, and NETs to accumulate in the precorneal tear film and cause ocular surface inflammation. The practical implication of our findings is the suggestion of new therapeutic interventions based on clearing eDNA, NETs, and their molecular components from the ocular surface, as well as inhibiting eDNA signaling pathway gene expression. These findings have been published in *Investigative Ophthalmology and Visual Sciences*¹⁹, and were quoted as a new insight into dry eye inflammation by an editorial comment in the same publication.²⁰ These findings underpin the basis of this research protocol.

1.2 Agent

The agent used in this study is recombinant human deoxyribonuclease I (rhDNase1). This agent is FDA approved for human use and is marketed as Pulmozyme (Genentech, San Francisco, CA). Pulmozyme is a sterile, clear, colorless, highly purified solution of recombinant human deoxyribonuclease I (rhDNase1). The protein is produced by genetically engineered Chinese Hamster Ovary (CHO) cells containing DNA encoding for the native human protein, deoxyribonuclease I (DNase1). Fermentation is carried out in a nutrient medium containing the antibiotic gentamicin, 100–200 mg/L. However, the presence of the antibiotic is not detectable in the final product. The product is purified by tangential flow filtration and column chromatography. The purified glycoprotein contains 260 amino acids with an approximate molecular weight of 37,000 daltons. The primary amino acid sequence is identical to that of the native human enzyme.

Each Pulmozyme single-use ampule has 2.5 mL of the solution. The aqueous solution contains 1.0 mg/mL dornase alfa (0.1%), 0.15 mg/mL calcium chloride dihydrate and 8.77 mg/mL sodium chloride. The solution contains no preservative. The nominal pH of the solution is 6.3.

Pulmozyme is approved by FDA for use in humans. Daily administration of Pulmozyme® (dornase alfa) Inhalation Solution in conjunction with standard therapies is indicated in the management of cystic fibrosis patients to improve pulmonary function. In patients with an FVC \geq 40% of predicted, daily administration of Pulmozyme has also been shown to reduce the risk of respiratory tract infections requiring parenteral antibiotics.

When 2.5 mg Pulmozyme was administered by inhalation to eighteen CF patients, mean sputum concentrations of 3 µg/mL DNase were measurable within 15 minutes. Mean sputum concentrations declined to an average of 0.6 µg/mL two hours following inhalation. Inhalation of up to 10 mg TID of Pulmozyme by 4 CF patients for six consecutive days, did not result in a significant elevation of serum concentrations of DNase above normal endogenous levels.^{21,22} After administration of up to 2.5 mg of Pulmozyme twice daily for six months to 321 CF patients, no accumulation of serum DNase was noted. Pulmozyme, 2.5 mg by inhalation, was administered daily to 98 patients aged 3 months to ≤ 10 years, and bronchoalveolar lavage (BAL) fluid was obtained within 90 minutes of the first dose. BAL DNase concentrations were detectable in all patients but showed a broad range, from 0.007 to 1.8 µg/mL. Over an average of 14 days of exposure, serum DNase concentrations (mean ± s.d.) increased by 1.3 ± 1.3 ng/mL for the 3 months to < 5 year age group and by 0.8 ± 1.2 ng/mL for the 5 to ≤ 10 year age group. The relationship between BAL or serum DNase concentration and adverse experiences and clinical outcomes is unknown.

1.3 Preclinical Data

We performed experiments that showed that eDNA, NETs, and neutrophils were present on the ocular surface in DED patients and abundant in mucoid films. NETs consisted of eDNA, histones, cathelicidin, and neutrophil elastase. Tear fluid nuclease activity was significantly decreased in DED patients, whereas the amount of eDNA on the ocular surface was significantly increased. Expression of genes downstream of eDNA signaling, such as TLR9, MyD88, and type I interferon, as well as the inflammatory cytokines interleukin-6 and tumor necrosis factor alpha, was significantly increased in DED patients. Therefore, based on our findings we conclude that extracellular DNA production and clearance mechanisms are dysregulated in DED.¹⁹ Nuclease deficiency in tear fluid allows eDNA and NETs to accumulate in precorneal tear film and results in ocular surface inflammation. These findings point to novel therapeutic interventions in severe DED based on clearance of eDNA, NETs, and other molecular components from the ocular surface. DNase I, an enzyme which selectively cleaves DNA, is one such therapeutic intervention. Our data showed that DNase I is naturally present in the lacrimal glands and tear fluid in humans. The concentration in tear fluid is 3.14 ng/ml. This concentration is similar to that in serum. Serum tear eye drops (containing DNase I) have been topically applied to the eye with significant therapeutic benefit to patients with severe dry eyes. To date there are no reports of direct application of DNase I to the eye.

1.4 Clinical Data to Date

To date, there is no published report of applying the study drug to the ocular surface as eye drops. However, eyes do get exposed to the study drug when it is administered to the lungs via a face mask. Eye exposure to nebulized drug is unavoidable.^{23,24} To date, no serious ocular side effects have been reported while using nebulized study drug, other than conjunctivitis in 0.4% patients, which was very mild and did not warrant drug discontinuation.²⁵ This is in contrast to other nebulized drugs such as albuterol (causes pupil dilatation and angle closure glaucoma) and steroids (causes cataracts and increase in eye pressure).²⁶⁻²⁸ Therefore, almost certainly, the eyes are exposed to study drug but there are no reports of serious adverse effects.

Further, we have used the study drug off label as a mucolytic agent to treat patients with recalcitrant filamentary keratitis to dissolve excessive mucus. In this patient, other mucolytics had failed (eg.- acetylcysteine eye drops). Use of the study drug (0.1% four times a day for one month) did not cause any symptoms of ocular discomfort (burning, irritation or soreness) and no adverse side effects were observed (ocular inflammation or eye pressure changes). Although the IRB application study involves a

different patient population than the patient in which DNase1 eye drop was used, our anecdotal evidence suggests that the drug is well tolerated when applied topically to the eyes in this concentration (0.1%) and dose (four times a day).

Based on documented lack of adverse effects in eyes exposed to the nebulized drug and our anecdotal evidence that the drug is well tolerated when applied topically to the eyes, we do not expect any severe adverse events with ocular use of this drug.

1.5 Dose Rationale and Risk/Benefits

RhDNase1 1mg/ml (0.1%) will be applied topically to the ocular surface as eye drops. This is the same dose as is currently in use in patients with Cystic Fibrosis. The drug formulation is being used unchanged. This dose has been effective in reducing eDNA content when given to patients with cystic fibrosis. We do not expect any serious adverse events with this dose.

Drugs are routinely applied topically to the ocular surface as eye drops because of easy access to the ocular tissues and good drug bioavailability. Therefore, we will use rhDNase1 topically. We have chosen an eight week duration of therapy because we expect rhDNase1 to be used continually; eight weeks of treatment will give us better data to predict side effects during long-term use. Since rhDNase1 is not expected to increase the production of natural DNase I, but rather it only supplements it so that the imbalance of decreased tear fluid DNase I is corrected, we expect long-term use of rhDNase1.

RhDNase is currently used as an inhaled drug in adults and children with cystic fibrosis. Patients with cystic fibrosis have experienced a change in or loss of their voice, discomfort in the throat, chest pain, red watery eyes, rash, dizziness, fever, or runny nose. Most of these are systemic side-effects that are not generally seen with eye drops. These side effects are usually mild and short-lived.

The most likely discomforts a subject may experience by having RhDNase administered as eye drops are eye burning or irritation, red watery eyes, or feeling like there is something in the eye. To see how subjects react to the study medication, their first dose will be given by the investigator in the clinic itself. Afterwards subjects will be asked to rate tolerability of the drug on the Visual Analogue Scale. Also, at each) visit, subjects will be asked to complete the Visual Analogue Scale.

An allergic reaction to the drug cannot be predicted beforehand. In event of an allergic reaction, the drug will be stopped immediately, and symptoms will be managed appropriately depending on the severity of the reaction.

Restasis, a drug used to treat DED, is held during the 14 day wash-out period and over the entire course of the study. Holding Restasis may increase the subject's risk of worsening DED.

Participants randomized to the placebo have a risk of worsening dry eye disease. All study subjects will be encouraged to contact the research team immediately if they experience worsening of their DED.

No psychological, social, legal, or financial risk is expected from participating in the research.

There is always a risk of a loss of confidentiality.

2 Study Objectives

The objective of this study is to establish whether patients with Dry Eye Disease are able to tolerate receiving rhDNase1 0.1% eye drops four times a day for eight weeks (primary tolerability objective) and to investigate the preliminary efficacy of rhDNase1 topical eye drop solution 0.1% in treating Dry Eye Disease (primary efficacy objective).

3 Study Design

3.1 General Design

This will be a Randomized controlled trial, in which a total of 72 patients will be enrolled at 1 clinical site. Subjects will be randomly assigned to one of two groups (#1, #2), with at least 30 subjects per group. Group #1 will be given placebo (drug vehicle- eye drops without rhDNase 0.1%) and Group #2 will be given study drug (rhDNase 0.1%).

Patients with established Dry Eye Disease will be approached by a member of the research staff to determine if the patient might be interested in participating in a research study. If the patient is interested, the research staff member will describe the study. If the patient is willing to enter the study, the study will be discussed and the patient will be asked to sign the informed consent form. Consent will be obtained prior to screening to determine eligibility. Screening procedures include documentation of prior Dry Eye Disease. Eligible patients will be enrolled in the study.

All enrolled subjects will receive their first dose of test medication (placebo/ study drug) on study Day 1 in the doctor's office and after completion of the study assessments, will have the topical eye drops dispensed for self-administration. See Section 5.4 *Preparation and Administration of Study Drug* for details.

Subjects will be provided with diaries to record the time of each dose and will also be asked to record any adverse symptoms. In addition, they will be asked to make a note of any missed doses together with the reason for the omission. Subjects will return two weeks later on Day 14 for further study assessments, thereafter at 4 weeks, 6 weeks and 8 weeks (the last day of treatment), and again at 10 weeks after two weeks of no treatment with study drug for the final study assessments.

3.2 Primary Study Endpoints

Primary Efficacy End Point: The primary end points after 8 weeks of treatment include the following: (1) the change in the Ocular Surface Disease Index (OSDI) which is a patient subjective rating scale; the Clinical Global Impression (CGI) of change in dry eye disease symptoms (physician's rating) and Subject Global Assessment (SGA) of overall change from baseline (patient's rating) will serve as anchors to OSDI, as has been described previously²⁹; (2) a mean reduction in ocular surface staining as measured by Rose Bengal dye staining and (3) the change in ocular surface redness score OR slit-lamp examination.

Primary Tolerability End Point: The change in the test substance tolerance between Day 1 (post-dose) and at weeks 2, 4, 6 and 8.

3.2.1 Efficacy End Point: Ocular Surface Disease Index (OSDI)

The OSDI rating scale has twelve questions in three discrete areas, with responses rated on a five point scale. Subjects will complete this scale on Day 1 prior to first dose (Baseline), week 2, week 4, week 6, week 8 and week 10. The questions and scoring system are shown below:

HAVE YOU EXPERIENCED ANY OF THE FOLLOWING DURING THE LAST WEEK:

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

HAVE PROBLEMS WITH YOUR EYES LIMITED YOU IN PERFORMING ANY OF THE FOLLOWING DURING THE LAST WEEK:

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

HAVE YOUR EYES FELT UNCOMFORTABLE IN ANY OF THE FOLLOWING SITUATIONS DURING THE LAST WEEK:

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

3.2.2 Efficacy End Point: Clinical Global Impression (CGI)

At each visit, the physician (Principal Investigator) will use his clinical evaluation (all signs and symptoms taken together) to provide a global assessment of the patients' change in Dry Eye symptoms and signs. The CGI is as follows:

Question (to physician): In general, compared with the patients' dry eye symptoms and signs at baseline, how would you characterize his/ her overall signs and symptoms now?

The responses will be categorized on a seven point scale as follows:

- Marked worsening
- Moderate worsening
- Minimal worsening
- Unchanged
- Minimal improvement
- Moderate improvement
- Marked improvement

3.2.3 Efficacy End Point: Subject Global Assessment (SGA)

At each visit, the subjects will be asked to assess their overall change from baseline. The SGA is as follows:

Question 1 (to subject): Compared with your first visit, how are your dry eye symptoms now?

The responses will be categorized on a five point scale as follows:

- Much worse
- Worse
- About the same
- Improved
- Much improved

Question 2 (to subject): Compared with your first visit, how is the mucous strings or mucous discharge from your eyes now?

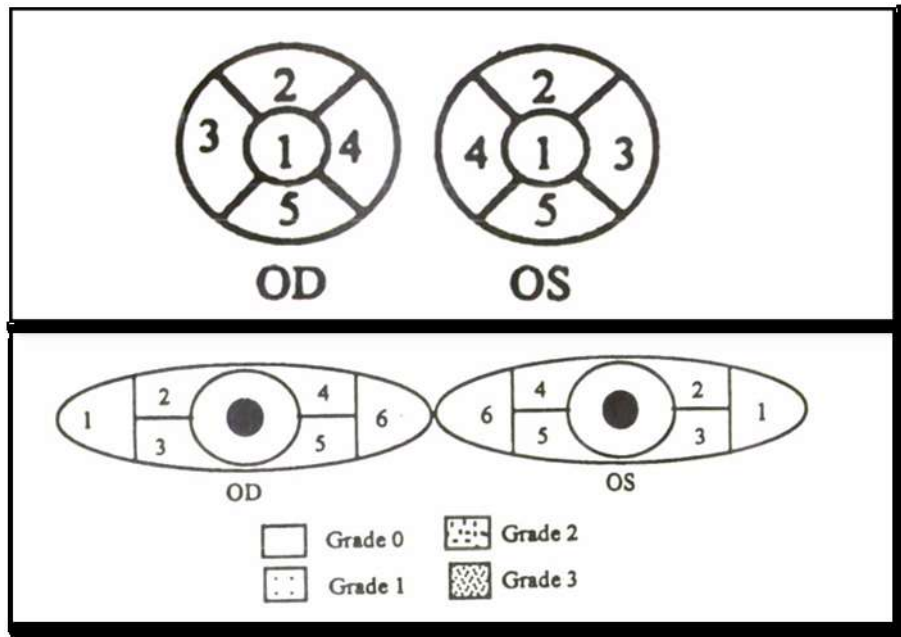
The responses will be categorized on a five point scale as follows:

- Much worse
- Worse
- About the same
- Improved
- Much improved

3.2.4 Efficacy End Point: Ocular surface Rose Bengal dye staining score

Ocular surface staining will be assessed using Rose Bengal dye. Saline moistened 1% Rose Bengal dye impregnated strips will be used to instill the dye on the inferior palpebral conjunctiva and scoring of corneal and conjunctival staining will be performed by a slit lamp examination after 15 seconds using the grading system described by the 1995 NEI workshop.³⁰ Corneal staining will be graded in 5 zones

and conjunctival staining will be graded in 6 zones. Each zone will be graded from 0 to 3 based on the density of punctate staining. The final staining score will be the sum of individual scores from all 11 zones (5 corneal, 6 conjunctival). The scoring pattern is represented below.



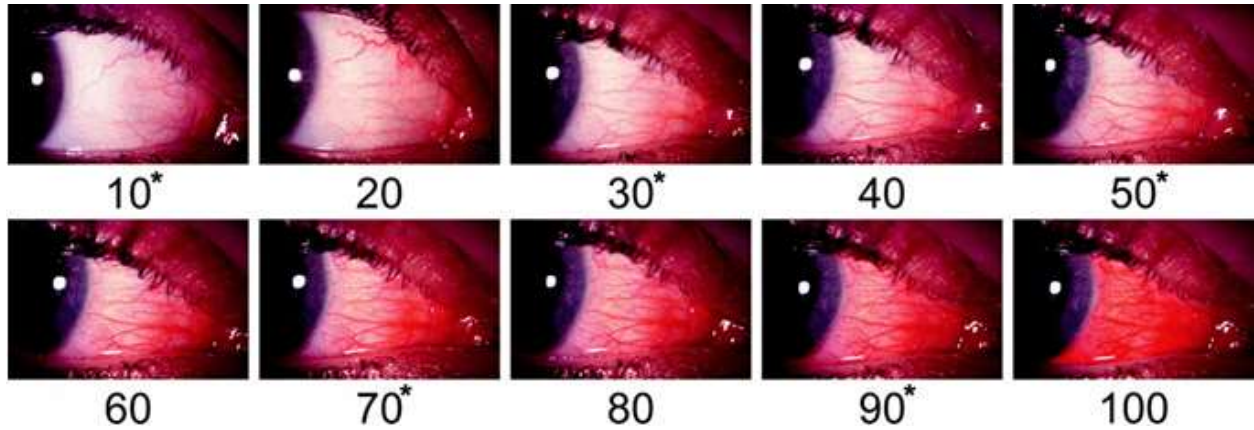
Complete ocular surface staining clearance means absence of any punctate or confluent staining with Rose Bengal dye defined as Rose Bengal staining score of 0.

3.2.5 Efficacy End Point: Ocular surface redness score

Ocular surface redness (nasal or temporal) will be assessed using the Validated Bulbar Redness grading scale (VBR).³¹ The VBR consists of a set of ten images illustrating different degrees of ocular surface redness (OR), ranging from normal to severe, and each image is assigned a value in an order of ascending severity. Colored copies of these images will be made using a single printer and constant settings and put up in all the examination rooms. Subjects will be examined by a slit-lamp at 10X magnification using direct diffuse illumination (slit fully opened, angled at 30°- 50° approximately; at half illumination intensity with rheostat set to maximum voltage) and the bulbar conjunctival injection of the subject's eye (nasal and temporal) will be compared to the reference images and graded accordingly. To maintain uniformity, all subjects will be graded by a single physician (Principal Investigator) under constant illumination conditions. The subjects will be asked to look at nasal or temporal fixation marks while the physician will examine the temporal or nasal bulbar conjunctivae, respectively.

Photographic anchors and their respective grades for ocular surface redness are shown below:

□



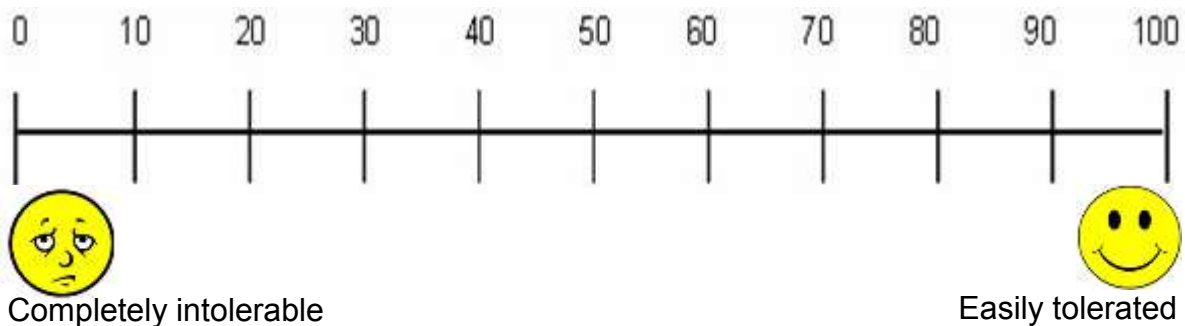
3.2.6 Tolerability End Point: Test Substance Tolerance (Visual Analogue Scale)

Subjects will assess their tolerance to the administration of the test medication (placebo/ study drug), utilizing a Visual Analog Scale (VAS). The VAS is a 100 mm horizontal line with verbal descriptors at either end. The VAS ratings will be completed after administration of the test medication on Day 1 (post-dose), week 2, week 4, week 6 and week 8. Subjects will place a single slash mark across the horizontal line between the end labeled “completely intolerable” (0 mm) and “easily tolerable” (100mm). The VAS rating is as follows:

Please rate the degree of comfort or lack of comfort associated with administering the eye drop by making one slash mark on the line below:

Visual Analogue Scale

On the scale of 0 to 100 seen below, please mark where you would rate your tolerability to administration of the test drug.



3.3 Secondary Endpoints

The secondary study endpoints will include:

1. Change in tear secretion as measured by Schirmer I test
2. The proportion of eyes achieving complete ocular surface staining clearance after treatment
3. Visual acuity change
4. Change in frequency of administration of artificial tears or concomitant eye drops
5. Change in number of corneal filaments (slit-lamp examination)

Corneal filaments are mucous tags that are adherent to the surface of cornea. The number of such mucus tags will be counted on each clinical examination (slit-lamp examination).

3.4 Primary Safety Endpoints

Safety assessments include Vital Signs, recording of all complications and adverse events, as well as ophthalmic exam findings. All ocular and non-ocular adverse events will be assessed for severity and relationship to the investigational product.

Primary safety endpoint:

- The proportion of subjects at week 8 who were able to successfully complete a full eight weeks of therapy with topical administration four times per day (q.i.d.)

Secondary safety endpoint:

- All adverse events reported, whether deemed related to treatment, or not.
- Clinically significant changes in vital signs or ophthalmic examination from baseline.

3.4.1 Vital Signs

Vital signs will be obtained and recorded at the Day 1 Visit, prior to the first administration of the test medication (placebo/ study drug) and on week 2, week 4, week 6, week 8 and week 10. The following vital signs will be measured: 1) blood pressure measurements (mm Hg) will be taken while the subject is relaxed in a sitting position for at least 3 minutes with the arm at heart level. 2) Heart rate will be measured via auscultation of the heart or palpation of a peripheral pulse and will be recorded in beats per minute (bpm). 3) Oral temperature will be recorded in degrees Fahrenheit (^oF). Subjects with an oral temperature less than (\leq) 99.6^oF (37.4^oC) may continue.

Clinically significant negative changes from baseline will be recorded on the adverse event forms.

3.4.2 Ophthalmic Examination

At all visits, the Investigator will conduct a complete undilated examination of the eyes using a binocular slit lamp. The Investigator will examine the tear film, eye lids, lashes, bulbar and palpebral conjunctiva, upper and lower lid puncta, cornea, anterior chamber, iris, lens, and anterior vitreous. Specific signs that will be recorded include: lacrimal sac area erythema, swelling or tenderness; froth or debris or mucous strands in tear film; eyelid hyperemia; punctal hyperemia or atresia; conjunctival/ episcleral hyperemia; papillary or follicular conjunctival reaction; chemosis, episcleral edema; superficial punctate keratopathy, corneal scar, corneal neovascularization; presence and number of corneal filaments, presence or absence of mucoid films, anterior chamber cell, flare or KPs; pupil shape abnormalities,

anterior or posterior synechiae, iris neovascularization; lenticular opacities; vitreous cells or pigment. Conjunctival hyperemia (ocular surface redness) will be graded at each visit using the VBR grading system as explained in section 3.2.5. Measurements at first and last visit will include: visual acuity (BCSVA), manifest refraction and intraocular pressure measurement. Clinically significant changes from baseline examination will be recorded on the adverse event forms.

4 Subject Selection and Withdrawal

4.1 Inclusion Criteria

Patients will be eligible for the study if all of the following criteria are met:

1. Aged 18 years or older.
2. Capable of giving informed consent and does provide informed consent.
3. Documented Dry Eye Disease for at least 6 months.
4. Schirmer I <10
5. Corneal/ conjunctival (Rose Bengal) staining ≥ 1
6. Ocular symptoms must be considered as annoying or activity limiting (OSDI ≥ 13 ; mild).
7. Women must be post-menopausal ≥ 1 year, or surgically sterilized. If not, a negative urine pregnancy test is required within 14 days of receiving her first dose of test medication (placebo/ study drug) along with definite evidence of contraceptive use during the duration of the study. Women of reproductive age should use a method of birth control that is acceptable to the subject and the study doctor. This may include oral contraceptive pills, birth control implants, barrier methods or abstinence. If a subject mentions she suspects she may be pregnant after being enrolled, another pregnancy test will be administered. If the test is positive, she will be discontinued from the study immediately.

4.2 Exclusion Criteria

Subjects will not be eligible for the study if any of the following criteria are met:

1. Allergic to rhDNase1 or any similar products, or excipients of rhDNase1 eye drops 0.1%.
2. Receiving or have received within 30 days any experimental systemic medication.
3. Active ocular infection or ocular allergies.
4. Any history of eyelid surgery or ocular surgery within the past 3 months.
5. Corneal epithelial defect larger than 1 mm² in either eye.
6. The use of topical cyclosporine or corticosteroids within 2 weeks of enrollment
7. Are homeless.
8. Have active drug/alcohol dependence or abuse history.

Except for a 2-week washout period of topical corticosteroids and topical cyclosporine before enrollment, participants will be permitted to continue their other chronic treatments, including the use of artificial tears, eyelid massage, or warm compresses.

4.3 Subject Recruitment and Screening

Potential subjects will be recruited from the clinical practice of the principal investigator (PI) at the time of their routine eye examination visit. The clinical practice is located in the Illinois Eye and Ear Infirmary, Department of Ophthalmology and Visual Sciences, University of Illinois at Chicago. Subjects will

include patients who have been diagnosed with dry eye in the investigator's eye clinic (cornea clinic or the comprehensive eye clinic), in the Illinois Eye and Ear Infirmary. Patients may also be referred to the PI's clinic by other Ophthalmology physicians at UIC, and Ophthalmology physicians at Loyola and North-Western Universities. The referring physicians shall inform the patients briefly about the study and provide them with the recruitment flier/ information sheet (enclosed), but no study related procedures (including screening and recruitment) will be performed at any location outside the PI's clinic at UIC (Illinois Eye and Ear Infirmary). Even the patients referred to the PI's clinic for possible enrollment in the study, will be screened and, if eligible, recruited in the study only at the PI's clinic. The physician letter (requesting for referrals for the study by the PI), and the information sheet that will be given to the subjects are attached.

Patients with established Dry Eye Disease (for at least 6 months, with Schirmer I < 10 and annoying or activity limiting visual symptoms) will be approached by a member of the research staff to determine if the patient might be interested in participating in a research study. If the patient is interested, the research staff member will describe the study. If the patient is willing to enter the study, the study will be discussed and the patient will be asked to sign the informed consent form. Consent will be obtained prior to screening to determine eligibility. Screening procedures include documentation of prior Dry Eye Disease as well as other assessments as detailed in section 6.5.1. Eligible patients will be enrolled in the study.

4.4 Early Withdrawal of Subjects

4.4.1 When and How to Withdraw Subjects

Subjects have the right to withdraw from the study at any time, for any reason, without jeopardizing their medical care. Where possible, subjects will be followed for safety and encouraged to return for follow-up visits for any unresolved safety events.

The IRB and Investigator also have the right to withdraw subjects from the study for the following reasons: when continuation may jeopardize the health of the subject, protocol violations, adverse events or concurrent conditions, administrative or other reasons.

4.4.2 Data Collection and Follow-up for Withdrawn Subjects

If a subject withdraws from the study prior to 8 weeks, the subject will be asked to complete the procedures outlined in the 10 week visit as well as the Test Substance Tolerance scale from 8 weeks, as soon as possible. Subjects who voluntarily withdraw from the study between 8 weeks and 10 weeks will be asked to complete procedures outlined in the 10 weeks visit as soon as possible. Subjects who are withdrawn due to adverse events will be followed at least until resolution or stabilization of the adverse event.

If the subject remains in the study for safety evaluation, follow-up visits will be scheduled according to the schedule of visits and procedures found in the synopsis.

5 Study Drug

5.1 Description

The study drug, rhDNase1, will be supplied as a 1 mg/ml (0.1%) solution for administration as topical eye drops. The control group will receive drug vehicle eye drops as placebo.

Each subject will receive either 0.1% rhDNase1 (study drug) or drug vehicle (placebo) solution, as a single eye drop in each eye four times a day (QID) for eight weeks. Except for the first dose on Day1, subjects will self-administer the test medication eye drops at home.

Subjects will not be charged for the test medication in any way (neither the cost of the medication nor its dispensing cost).

5.2 Treatment Regimen

Study drug group- rhDNase1, 1mg/ml (0.1%) eye drops will be applied to both eyes q.i.d for 8 weeks. Control group - drug vehicle eye drops will be applied to both eyes q.i.d for 8 weeks. For either group, the patient will be instructed to instill the first dose of the study medication in the morning at approximately 8 a.m., and then the remaining doses at approximately 4 hourly intervals. Therefore, doses will be scheduled at approximately 8 a.m., 12 noon, 4 p.m. and 8 p.m.

5.3 Method for Assigning Subjects to Treatment Groups

This Randomized placebo-controlled trial will have two study groups. Subjects will be randomly assigned to one of two groups (#1, #2). Group #1 will receive placebo (drug vehicle; control group) and Group #2 will receive study drug (rhDNase 0.1%; test group). We will use a computer-based random code generator (Research Randomizer; <http://randomizer.org/>) to generate 1 set of 72 non-unique, unsorted numbers with a range from 1 to 2 representing the group number (#1 is control group, #2 is test group). Each subject will be assigned a study identification (ID) number at screening, eg. subject #1, subject #2, subject #3 and so on. Based on the randomizer generated table, subject #1 will receive either placebo (#1 group) or study drug (#2 group). This will be repeated for each subject. For reproducibility purpose, we will document the final randomization schedule and the random SEED number used to generate the schedule. Randomization will be performed by the Illinois Eye and Ear Infirmary's pharmacy, and neither participants nor research staff will be aware of the assigned treatments. The person conducting the randomization will remain masked as well.

The study identification (ID) number will be used on all study-related documents. To maintain confidentiality, the subject's name will not be recorded on any study document other than the informed consent form. The drug vial number will be linked to the patient identification number.

5.4 Preparation and Administration of Study Drug

The study medications will be stored, packaged and dispensed from the UIC Eye and Ear Infirmary (EEI) Pharmacy. The study medications will be dispensed in sterile eye droppers of 3 ml volume (each containing 400- 500µl of the study medications), which will be used as single-dose applications. One drop of the drug/ placebo solution will be administered to each eye. Therefore 4 eyedroppers will be required per day. At each visits, subjects will receive 56 sterile multi-dose eye droppers that will be used as single-dose applications. Prepared eye droppers will be placed in a dark (brown) colored zip-lock packet before being dispensed to the subject. The medications will need to be stored in a refrigerator (4 °C), away from direct strong light.

Instructions for Drug Use:

1. Wash your hands thoroughly with soap and water.
2. Check the dropper tip to make sure that it is not chipped or cracked.
3. Avoid touching the dropper tip against your eye or anything else – eye drops and droppers must be kept clean.
4. While tilting your head back, pull down the lower lid of your eye with your index finger to form a pocket.
5. Hold the dropper (tip down) with the other hand, as close to the eye as possible without touching it.
6. While looking up, gently squeeze the dropper so that a single drop falls into the pocket made by the lower eyelid. Remove your index finger from the lower eyelid.
7. Close your eye for 2 to 3 minutes and tip your head down as though looking at the floor. Try not to blink or squeeze your eyelids.
8. Place a finger on the tear duct and apply gentle pressure.
9. If you are to use more than one drop in the same eye, wait at least 5 minutes before instilling the next drop.
10. Do not reuse the dropper after use. Use another dropper for next dose.

The subject should repeat the above procedures for the other eye to demonstrate to the Investigator or designee that they are able to perform the drug administration satisfactorily.

Subjects will be instructed to perform these steps on each administration of the study medication.

Instructions for use will be included in the zip-lock packet with the study medication and site personnel will ensure that these instructions are given to the patient.

5.5 Subject Compliance Monitoring

Subjects will receive their first dose of study medication on study Day 1 in the doctor's office and after completion of the study assessments will have the topical eye drops dispensed for self-administration.

Subjects will be provided with diaries to record the time of each dose and will also be asked to record any adverse symptoms. In addition, they will be asked to make a note of any missed doses together with the reason for the omission. Subjects will be asked to bring their diaries with them at the 2, 4, 6 and 8 week visits. Diaries will be reviewed with the subject by a member of the research team at each visit. Additionally, subjects will be asked to bring back the used and unused drug eye droppers at each study visit. Participants will be asked to return the unused eye droppers each study visit as a method to determine compliance.

5.6 Prior and Concomitant Therapy

Prior medications are defined as all medications taken within 30 days prior to Day 1, whether there is continued use or not. Concomitant medications must be identified in the patient's medical record, including all lubricants administered for Dry Eye Disease. These medications will be recorded in the case report form (CRF).

• For each medication taken, the following information will be collected:

1. Medication trade name
2. Eye that was treated, if applicable
3. Indication for which the medication was given

4. Date started
5. Date stopped
6. Dose of medication used.

There will be a 2-week washout period of topical corticosteroids and topical cyclosporine before enrollment and these medications are prohibited throughout the study. Other than these, participants will be permitted to continue their chronic DED treatments which include the use of artificial tears, eyelid massage, or warm compresses. The number of drops and frequency must be recorded in the patient diary provided.

- The use of any investigational agent during past 30 days is prohibited.

There is only one FDA approved treatment currently available to treat patients with Keratoconjunctivitis Sicca (dry eye disease)- Cyclosporine 0.05% (marketed as Restasis). The FDA approved indication for Restasis use is the following: “to increase tear production in patients whose tear production is presumed to be suppressed due to ocular inflammation associated with keratoconjunctivitis sicca”. Restasis will be withheld during the course of this study. This is necessary as the proposed mechanism of action of rhDNase in dry eyes is to reduce inflammation on the surface of the eye. Restasis can also reduce inflammation albeit via a different mechanism. Thus, because of over lapping anti-inflammatory actions, it may not be possible to attribute any observed clinical benefit to rhDNase treatment if Restasis is used concurrently. This approach has been used in other clinical trials that have investigated the use of an anti-inflammatory agent in dry eye disease.³²

It is ethical to withhold Restasis because, as its FDA approved indication suggests, the clinical benefit of using Restasis is increased production of tears. While receiving DNase1, patients will continue to receive artificial tears as needed. Therefore, although the patients may not have an increase in tear production if Restasis is withheld, the ocular surface will receive sufficient lubrication and hydration due to the use of artificial tears.

Holding Restasis may increase the risk of worsening of DED during the wash-out period and during the course of the study. However, because artificial tears will be used during the wash out period as well as during the course of the study as needed, we do not expect any significant clinical worsening during the study. We do however expect the frequency of use of artificial tears to go up during the wash out period and during the course of the study.

The subjects will be monitored frequently (at 2 weekly intervals) to ensure that they are not subjected to any undue risks during the wash out period or during the course of the study. Additionally, they will be warned of the possible signs and symptoms of clinical worsening of DED, and advised to contact the research team immediately in case any of those symptoms occur. Subjects will also be encouraged to contact the research team in case they experience any ocular discomfort during the wash out period or during the course of the study. The subject's condition will be monitored by the physician (Principal Investigator) at each study visit, as well as at any interim visit (in case of adverse symptoms, as mentioned above). Any worsening of DED or any adverse event due to the study drug will be recorded. In case of clinical worsening, based on the individual subject's clinical condition, one or more of the following therapeutic decisions may be implemented: (1) Increasing the frequency of use of artificial tears, (2) Re-starting the use of anti-inflammatory therapy (Restasis/ corticosteroids), (3) Withdrawal of the use of study drug (if worsening occurs during the course of the study). The decision will be made by the physician (Principal Investigator) based his clinical judgment as per the individual subject's clinical condition. If additional anti-inflammatory therapy (Restasis/ corticosteroids) is instituted during the wash-out period, the subject will be excluded from the study. Also, if additional anti-inflammatory therapy (Restasis/ corticosteroids) is instituted during the course of the study and/or the study drug is

withdrawn, the subject will be excluded from the study. After any clinical worsening is noted, the subjects will be followed more closely (weekly) until complete resolution of symptoms and return to the subject's previous baseline.

5.7 Rescue Plan

The research staff, including the PI, will be masked to randomization, thus will not be aware if a particular subject receives study drug or placebo. Subjects will be monitored by the Principal Investigator at each study visit. Any worsening of DED or any adverse event (AEs) will be recorded and in the case of AEs followed to resolution. In case of clinical worsening/ adverse event(s), based on the individual subject's clinical condition, one or more of the following therapeutic decisions may be implemented:

- (1) Increasing the frequency of artificial tears use,
- (2) Re-starting the use of anti-inflammatory therapy (Restasis/ corticosteroids),
- (3) Discontinue the study drug.

The decision will be made by the Principal Investigator based on his clinical judgment and the individual subject's clinical condition. If additional anti-inflammatory therapy (Restasis/ corticosteroids) is instituted and/or the study drug is discontinued, the subject will be withdrawn from the study. If an adverse event is severe enough to discontinue the subject from the study, the PI may decide to break the subject's randomization code if it seems relevant to the treatment of his/her ocular condition at that time. He/ She will receive the treatment required for his/ her eye condition as per established clinical guidelines.

5.8 Packaging

The study medications will be dispensed in sterile multi-dose eye droppers of 3 ml volume, which will be used as single-dose applications. One drop of the placebo/ drug solution will be administered to each eye. Therefore 4 eye droppers will be required per day. At each visit, subjects will receive 56 sterile multi-dose eye droppers that will be used as single-dose applications. The eye droppers will be placed in a dark (brown) colored packet before being dispensed to the subject. A label with abbreviated information will be placed on each eye dropper. The zip-lock packet will include the subject's name, stage of visit, instructions for drug use and storage and the drug expiration date. The label will also include the study name (abbreviated) and a statement that the drug is **investigational** for use only in this research study." The first dose will be administered to the patient by the researcher from one of the eye droppers that will be dispensed to the subject at the first treatment visit (visit 2, day 1). No separate packing will be done for the study medication to be used in the MD's office. The subjects will receive the week's remaining doses in a dark packet with an ice pack to take home. Fifty-six sterile eye droppers will be dispensed at each visit.

5.9 Receiving, Storage, Dispensing and Return

5.9.1 Receipt of Drug Supplies

The UIC Investigational Drug Service (IDS) will order the study drug. The study drug will be stored in the Taylor Street/ EEI pharmacy and dispensed to subjects as needed.

5.9.2 Storage

Study medication will be stored at EEI pharmacy until such time as a subject visit is scheduled. The study medication will be directly dispensed to the subject from the EEI pharmacy on each treatment

visit, except for the first dose that is administered in the clinic under the supervision of research personnel. The study medication will not be stored in the MD's office, except when a subject receives his/her first dose, when the medication may be kept in the MD's office for a maximum of 2- 3 hrs in the ice pack provided by the EEI pharmacy.

5.9.3 Dispensing of Study Drug

At the first treatment visit (day 1), the first study medication dose will be administered to the subject in the clinic and eye droppers sufficient to last for 2 weeks will be given to them to take home. The subjects will be asked to return the used and unused eye droppers at the follow- up visits. We will then retrieve the previously -dispensed eye droppers and a fresh 2 week supply will be dispensed by the pharmacy. This will continue from week 2 to week 8. No new drug eye droppers will be given on the 6th (week 8) visit.

5.9.4 Return or Destruction of Study Drug

At the completion of the study, there will be a final reconciliation of drug ordered/ received, drug consumed, and drug remaining. Any discrepancies will be investigated, resolved, and documented. The used drug eye droppers will finally be disposed by the pharmacy according to the pharmacy standard protocols.

6 Study Procedures

6.1 Patient Recruitment and Screening

Prior to recruitment of any patients into the study, written approval of the protocol and informed consent will be obtained from the Institutional Review Board (IRB).

Potential subjects will be recruited from the clinical practice of the investigator at the time of their eye examination visit. The clinical practice is located at the Department of Ophthalmology and Visual Sciences, Eye and Ear Infirmary, 1855 W. Taylor Street, Chicago IL 60612. Patients with established Dry Eye Disease (for at least 6 months with annoying or activity limiting visual symptoms) will be approached by a member of the research staff to determine if the patient might be interested in participating in a research study. If the patient is interested, the research staff member will describe the study. If the patient is willing to enter the study, the study will be discussed and the patient will be asked to sign the informed consent form. Consent will be obtained prior to screening to determine eligibility. Subjects will be screened for eligibility, as per the inclusion/ exclusion criteria, and as detailed in section 6.5.1. Eligible patients will be enrolled in the study.

6.2 Assignment of Patient Identification

A study identification (ID) number will be assigned to each subject at screening. This study ID number will be used on all study-related documents. To maintain confidentiality, the subject's name will not be recorded on any study document other than the informed consent form. The master code list will link the subject MRN to the study ID number given to each subject. The master code list will be stored on the desktop in PI's OFFICE in Lions of Illinois Eye Research Institute (LIERI). The data collected and master code list will be accessible only to the PI and the research team involved in this project. The desktops will be password protected as well. Data will not be shared over the internet and will remain password protected. Confidentiality will be maintained. Data will be de-identified once study is completed and for subjects determined to not meet eligibility criteria or who later decline participation in the consent process.

6.3 Screen Failure

A record of screen failures and the reasons for non-eligibility to the study will be maintained.

6.4 Patient Enrollment

Patients meeting the enrollment criteria (see Sections 4.1 and 4.2) will be eligible for the study.

6.5 Study Assessments

The following detailed procedures are performed at the designated clinic visit. All results will be documented on the subject's medical/research charts, source documents, and CRFs as required. All ophthalmic procedures will be performed on both eyes.

6.5.1 Visit 1 Screening

Day -18 to 0

After obtaining informed consent, the following assessments will be performed within fourteen days prior to the subject receiving the first dose of study medication:

- Demographic information including: birth date, gender, race or ethnic origin.
- Medical History including prior medication use and prior procedures: Medical history will be obtained by interviewing the subject and will include a review of the following systems: cardiovascular, dermatologic, gastrointestinal, genitourinary, musculoskeletal, neurologic and respiratory. An allergic history (including medications and food), substance abuse history (including alcohol) and a history of medication use (including prescription, OTC, and herbal products) during the past 30 days will also be completed.
- Ophthalmic and Dry Eye Disease history including: date when the Dry Eye Disease began, verification that the subject has had Dry Eye Disease for at least 6 months, medications used by the subject to treat Dry Eye Disease, previous procedures to treat Dry Eye Disease.
- Ophthalmic Examination (slit lamp examination, Ocular surface redness score, Rose Bengal staining, and a Schirmer 1 test)
- Pregnancy test (urine), if applicable. Women of reproductive age will be asked to use a method of birth control that is acceptable to the subject and the study doctor. This may include oral contraceptive pills, birth control implants/shots or patches, barrier methods or abstinence. Women of reproductive age will not be included in the study if they refuse to use any birth control measure, including abstinence.

Patients currently treating DED with corticosteroids and/or Restasis will stop these treatments during a wash-out period (2 weeks before the start the study drug) and during the whole study. The study doctor will provide advice about decreasing DED symptoms with artificial tears, eyelid massage, or warm compresses.

If the patient agrees to enter the study, the first treatment visit will be scheduled. If a washout period is needed, Visit 2 will be after 2 weeks (\pm 4 days). If a washout period is not necessary, Visit 2 can be scheduled any time in the next 2 weeks after the Screening Visit.

6.5.2 Visit 2 Day 1 (*Randomization and First treatment visit*)

Prior to first dose (Baseline)

- Vital Signs (blood pressure taken while subject is relaxed in a sitting position for at least 3 minutes, pulse, temperature, and height and weight (at this visit only).
- Baseline Ophthalmic Examination (slit lamp examination, Ocular surface redness score)
- BSCVA (Snellen)
- OSDI
- Clinical Global Impression, Subject Global Assessment
- Record changes in concomitant medication
- Adverse events since screening visit.

Investigator/designee administers first dose

Post-Dose

- Visual Analogue Scale
- Subjects will be trained on how to self-administer the study eye drops and be given a sufficient supply to last for 2 weeks to take home.
- Subjects will receive a study diary on which to record the day/time of each dose and any adverse effects.
- Post dose evaluation for any adverse effects.

6.5.3 Visit 3: *Week 2 (± 2 days)*

Treatment Visit (after any dose for that day)

- Vital Signs
- Ophthalmic Examination (slit lamp examination, Ocular surface redness score)
- Physical Inspection of the Nose and Oropharynx
- OSDI
- Visual Analogue Scale
- Clinical Global Impression, Subject Global Assessment
- Review the subject's diary and record changes in concomitant medication, deviations from drug schedule and adverse events.
- Subjects will be given study medication, sufficient to last for 2 weeks, to take home.

6.5.4 Visit 4: *Week 4 (± 2 days)*

Treatment Visit (after any dose for that day)

- Vital Signs
- Ophthalmic Examination (slit lamp examination, Ocular surface redness score)
- Physical Inspection of the Nose and Oropharynx
- OSDI
- Visual Analogue Scale
- Clinical Global Impression, Subject Global Assessment
- Review the subject's diary and record changes in concomitant medication, deviations from drug schedule and adverse events.

- Subjects will be given study medication, sufficient to last for 2 weeks, to take home.

6.5.5 Visit 5: Week 6 (\pm 2 days)

Treatment Visit (after any dose for that day)

- Vital Signs
- Ophthalmic Examination (slit lamp examination, Ocular surface redness score)
- Physical Inspection of the Nose and Oropharynx
- OSDI
- Test Substance Tolerance
- Clinical Global Impression, Subject Global Assessment
- Review the subject's diary and record changes in concomitant medication, deviations from drug schedule and adverse events.
- Subjects will be given study medications, sufficient to last for 2 weeks, to take home.

6.5.6 Visit 6: Week 8 (\pm 2 days)

Treatment Visit (after any dose for that day)

- Vital Signs
- Ophthalmic Examination (slit lamp examination, Ocular surface redness score, Rose Bengal staining, and a Schirmer 1 test)
- Physical Inspection of the Nose and Oropharynx
- OSDI
- Test Substance Tolerance
- Clinical Global Impression, Subject Global Assessment
- Review the subject's diary and record changes in concomitant medication, deviations from drug schedule and adverse events.

6.5.7 Visit 7: Week 10 (\pm 2 days)

Follow-Up Visit

- Vital Signs
- Ophthalmic Examination (slit lamp examination, Ocular surface redness score)
- Physical Inspection of the Nose and Oropharynx
- BSCVA (Snellen)
- OSDI
- Clinical Global Impression, Subject Global Assessment
- Review the subject's diary and record changes in concomitant medication and adverse events.

7 Statistical Plan

7.1 Sample Size Determination

Our intention is to have at least 30 subjects in each group (study drug/ vehicle) complete the study. This number is based on published reports of similar randomized clinical trials designed to determine

efficacy and tolerability of a test drug in Dry Eye Disease.³²⁻³⁴ A total of 72 subjects will be enrolled so that even if 20% of subjects are unable to complete the study, we will still remain with the intended number of subjects who complete the study (at least 30 subjects in each group). Subjects will be randomized to either of the two groups (#1, placebo; #2, study drug) as explained in section 5.3. However, all subjects who received at least two weeks of treatment with study eye drops and attended at least the first post-treatment (week 2) follow up visit will be included for analysis.

7.2 Statistical Methods

Descriptive statistics will be used for all primary and secondary endpoints, when appropriate.

We will define outcome measures on the basis of the change for each subject from baseline to each follow-up point, controlling for chance imbalances in the mean baseline values between treatment groups. A Wilcoxon Signed-rank test will be used for paired comparison of quantitative variables (OSDI score) between the baseline and each post-baseline time points. Chi-square test will be used to compare qualitative variables (Investigator's rating scale/ changes in concomitant medications) between the 2 groups. Two-sided $P < .05$ will be considered statistically significant for all comparisons. 95% Confidence interval will be provided for comparison between the investigational drug and placebo for the primary efficacy, the primary safety, and the secondary endpoints.

7.3 Subject Population(s) for Analysis

Subject population for analysis will include any subject enrolled in the study who received at least two weeks of treatment with study eye drops and attended at least the first post-treatment (week 2) follow up visit.

8 Safety and Adverse Events

8.1 Adverse Event Definitions

The following are specific definitions of terms guided by the International Conference on Harmonization (ICH) Guidelines for Good Clinical Practice (GCP) and the U.S. Code of Federal Regulations that apply to this section:

Adverse Event: Any untoward medical occurrence in a subject or clinical investigation subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment. An adverse event can be any unfavorable sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of the investigational product, whether or not considered related to the investigational product.

Subjects will be reminded to inform the study staff of any adverse effects that they have experienced or are experiencing after the first administration of study drug. In addition, subjects will record adverse events in their diary throughout the study. All reports of adverse events during the study will be recorded on an Adverse Event Case Report Form (CRF). The subject should not be prompted about any adverse events that may occur during this trial.

For each adverse event, the following information will be recorded on the subject's Case Report Form(s): onset date, end date or continues, intensity, duration, relationship to test patch, action taken, and outcome. If a subject experiences a serious adverse event (SAE), study staff may discontinue the subject from study participation. The study staff must notify the IRB within 24 hours of receipt of the information. The study staff will instruct the subject to notify the research facility should any adverse

event occur within 7 days of study completion. (For definitions of an AE and SAE, see below). Subjects who withdraw due to an adverse event may be replaced.

- **Serious Adverse Event:** An untoward medical occurrence that at any dose:
 1. results in death
 2. is life-threatening
 3. requires inpatient hospitalization or prolongs existing hospitalization
 4. results in persistent or significant disability/incapacity
 5. is a congenital anomaly/ birth defect
 6. requires medical or surgical intervention to prevent any of the occurrences noted above.

- **Life-threatening:** Any adverse drug experience in which the subject was at risk of death at the time of the event. It does not refer to an event which hypothetically might have caused death if it were more severe.

- **Unexpected adverse event:** Any adverse event, the specificity or severity of which is not consistent with the current Investigator's Brochure.

8.2 Classification of Adverse Events by Severity

All toxicities/adverse events will be graded according to the following definitions to code the intensity of the event.

Mild: Usually transient, requiring no special treatment, and does not interfere with the subject's daily activities.

Moderate: Traditionally introduces a low level of inconvenience or concern to the subject and may interfere with daily activities, but are usually relieved by simple therapeutic measures.

Severe: Causes an interruption of the subject's usual daily activity and traditionally required systemic drug therapy or other treatment.

Note: If the intensity of an adverse event changes, the event will be reentered as a separate event.

There is a distinction between the severity and the seriousness of an adverse event. Severity is a measurement of intensity; thus, a severe reaction is not necessarily a serious adverse event. For example, a headache may be severe in intensity, but would not be serious unless it met one of the criteria for serious adverse events listed previously.

8.3 Classification of Adverse Events by Relationship to Study Treatment

The relationship or association of the study medication to an adverse event, as causing or contributing to the adverse event, will be characterized as defined below:

Probable: The adverse event follows a reasonable temporal sequence from administration of the drug (including the course after withdrawal of the drug) and the possibilities of factors other than the drug, such as underlying disease, concomitant drugs or concurrent treatment, can be excluded.

Possible: The adverse event follows a reasonable temporal sequence from administration of the drug (including the course after withdrawal of the drug) and the possibility that drug involvement cannot be

excluded, e.g. existence of similar reports attributable to the suspected drug, its analog or its pharmacological effect. However, other factors such as underlying disease, concomitant drugs or concurrent treatment are presumable.

Not Related: The adverse event has no temporal sequence from administration of the drug, or it can be explained by other factors, including underlying disease, concomitant drugs or concurrent treatment.

8.4 Action(s) Taken

One or more of the following will be recorded by the Investigator for each adverse event:

- No action taken
- Discontinued study drug (Subject withdrawn due to this adverse event)
- Administered therapy
- Hospitalized subject (due to this adverse event)
- Other (specify) - includes tests, labs confirming reaction

8.5 Outcome

The status of each adverse event will be recorded as follows, if applicable: SAE: Indicates that the adverse event met the criteria of a serious adverse event (SAE) and the SAE was reported to the IRB. Caused Withdrawal: Indicates that the adverse event caused the subject's withdrawal from the study.

8.6 Adverse Event Reporting

All subjects who have been exposed to study drug will be evaluated for adverse events. Adverse events will be recorded starting after the first dose of study drug and continuing until the end of the study. All adverse events will be evaluated beginning with onset, and evaluation will continue until resolution is noted, or until the Investigator determines that the subject's condition is stable, whichever is earlier. The Investigator will take all appropriate and necessary therapeutic measures required for resolution of the adverse event. Any medication necessary for the treatment of an adverse event must be recorded on the concomitant medication case report form. If more than one distinct adverse event occurs, each event should be recorded separately. Procedures such as surgery should not be recorded as adverse events. However, the medical condition for which the procedure was performed should be reported if it meets the definition of adverse event as described previously.

8.7 Serious Adverse Event Reporting

All Serious Adverse Events (SAE) that occur during the course of the study, including death, which are unanticipated require reporting to the IRB within **5 business** days of the investigator becoming aware. Serious adverse events will be recorded starting after first dose of study drug and continuing until the end of the study. The minimum information to be provided includes:

1. Protocol Number
2. Initial reporter
3. Subject identification
4. Nature and date of the event/effect
5. Country of the event/effect
6. Severity of the event/effect
7. Reporting criteria
8. Narrative description of the event/effect

9. Outcome if known
10. Causal relationship to the investigational product
11. Additional and follow-up information as requested by the medical monitor.

Events requiring reporting to the IRB within **15 business days** of the investigator becoming aware include:

1. Local adverse events or problems that are unanticipated and, while not meeting the criteria of serious, indicate research is associated with a greater risk of harm to participants or others than previously known.
2. New information indicating an unexpected change to the risks or benefits of the research (i.e., an unanticipated problem).
3. Administrative hold by investigator, regulatory authorities or other entities.

8.8 In Case of an Emergency

In medical emergencies, the Investigator should use medical judgment and remove the subject from immediate hazard. The IRB should be notified as to the type of emergency and the course of action taken. The CRF and the source document for the subject must describe the departure from the protocol and state the reason.

8.9 Data Safety Management Plan

The study protocol will be reviewed and approved by the UIC IRB. Adverse events and compliance will be monitored. Research staff will be trained on the protocol requirements and data collection methods before completing study related procedures.

8.10 Study Oversight

The Study PI has primary oversight responsibility for this study. Sandeep Jain, MD is a board certified Ophthalmologist with an active practice in the area of Dry Eye Disease. He routinely takes care of patients with severe ocular surface disease. He's also the director of Dry Eye service at UIC. Therefore, he's well qualified to recognize the symptoms and clinical signs of an adverse event. Dr. Jain has been the PI of past and active IRB approved clinical studies and has monitored data related to those studies. Therefore, he has experience in data and safety monitoring.

The Principal Investigator and his research team are responsible for identifying adverse events. Safety monitoring will include careful assessment and appropriate reporting of adverse events. Subjects will be reminded to inform the study staff of any adverse effects that they have experienced or are experiencing after the first administration of study drug. Subjects will be provided with diaries to record at home the time of each dose and any adverse symptoms. All reports of adverse events during the study will be recorded on an Adverse Event Case Report Form (CRF). In addition, subjects will be asked to make a note of any missed doses together with the reason for the omission. A member of the research staff will review diary entries with the subject at each study visit. Subjects will be asked to bring back the left-over drug at each study visit. The amount of drug remaining in the used vial will also give an estimate of the compliance.

Accumulated safety and data information will be reviewed after 10 subjects complete the study. The research team will then evaluate whether it is safe to proceed with the study, and if the protocol or informed consent documents require revision based on that review.

9 Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e. that the subject is alive) at the end of their scheduled study period.

10 Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

10.1 Records Retention

It is the investigator's responsibility to retain study essential documents. Research file documents will be uniformly held indefinitely after the closure of the research file per UIC IRB requirements.

11 Ethical Considerations

This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted independent Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the IRB concerning the conduct of the study will be made in writing to the investigator. The study may not commence until IRB approval is granted.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. This consent form will be submitted with the protocol for review and approval by the IRB for the study. The formal consent of a subject, using the IRB-approved consent form, must be obtained before that subject undergoes any study procedure. The consent form must be signed by the subject or legally authorized representative, and the investigator-designated research professional obtaining the consent.

12 Study Finances

12.1 Funding Source

Departmental funds have been committed to the Corneal Neurobiology Laboratory. Also, Genentech is partially funding this research. For the release of funds, they will have access to some study data. We will email Genentech the enrollment updates and clinical visit logs at regular intervals. The data emailed to Genentech will be linked with a code. Only the PI, and Key Research personnel listed in Appendix P will have access to the linked code. PHI and sensitive identifiable data will not be shared via email with any entity. Genentech may also inspect records relevant to the study, to ensure compliance with the terms of agreement between the study PI and Genentech. Any inspection of study records by Genentech will be performed on site only (here at UIC).

12.2 Conflict of Interest

Any investigator who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must have the conflict reviewed by a properly constituted Conflict of Interest Committee with a Committee-sanctioned conflict management plan. All UIC investigators will follow the University conflict of interest policy.

12.3 Subject Stipends or Payments

\$30/ visit will be given to each patient on baseline visit and on the five subsequent visits afterwards till week 10 to offset to some extent their parking/ transportation expenses.

References

1. [No authors listed]. The definition and classification of dry eye disease: report of the Definition and Classification Subcommittee of the International Dry Eye Workshop Ocul. Surf. 2007;5 :75–92.
2. Stevenson W, Chauhan SK, Dana R. Dry eye disease: an immune-mediated ocular surface disorder. Arch Ophthalmol. 2012;130:90-100.
3. Pflugfelder SC, de Paiva CS, Li DQ, Stern ME. Epithelial-immune cell interaction in dry eye. Cornea. 2008 Sep;27 Suppl 1:S9-11.
4. Barabino S, Chen Y, Chauhan S, Dana R. Ocular surface immunity: homeostatic mechanisms and their disruption in dry eye disease. Prog Retin Eye Res. 2012;31:271-85.
5. Cenedella RJ, Fleschner CR. Kinetics of corneal epithelium turnover in vivo. Studies of lovastatin. Invest Ophthalmol Vis Sci. 1990;31:1957-62.
6. Hanna C, O'Brien JE. Cell production and migration in the epithelial layer of the cornea. Arch Ophthalmol. 1960;64:536-9.
7. Kunert KS, Tisdale AS, Gipson IK. Goblet cell numbers and epithelial proliferation in the conjunctiva of patients with dry eye syndrome treated with cyclosporine. Arch Ophthalmol. 2002;120:330-7.
8. Thoft RA, Friend J. The X, Y, Z hypothesis of corneal epithelial maintenance. Invest Ophthalmol Vis Sci. 1983;24:1442–1443.
9. Ladage PM, Jester JV, Petroll WM, et al. Vertical movement of epithelial basal cells toward the corneal surface during use of extended-wear contact lenses. Invest Ophthalmol Vis Sci. 2003;44:1056–1063.
10. Ren H, Wilson G. Apoptosis in the corneal epithelium. Invest Ophthalmol Vis Sci. 1996;37:1017–1025.
11. Zhang Q, Raoof M, Chen Y, Sumi Y, Sursal T, Junger W, Brohi K, Itagaki K, Hauser CJ. Circulating mitochondrial DAMPs cause inflammatory responses to injury. Nature. 2010 Mar 4;464(7285):104-7.
12. Pisetsky DS. The origin and properties of extracellular DNA: From PAMP to DAMP. Clin Immunol. 2012;144:32-40.
13. Tanioka H, Yokoi N, Komuro A, Shimamoto T, Kawasaki S, Matsuda A, Kinoshita S. Investigation of the corneal filament in filamentary keratitis. Invest Ophthalmol Vis Sci. 2009;50(3):3696-702.
14. Yusifov TN, Abduragimov AR, Narsinh K, Gasymov OK, Glasgow BJ. Tear lipocalin is the major endonuclease in tears. Mol Vis. 2008;14:180-8.
15. Junko F, Yoko H, Koji T, Yuing X, Norihito N, Shinji I, Kaori K, Hariuo T. Deoxyribonuclease I typing from tears. Shimane J Med Sci. 2004;22:44-9.
16. Napirei M, Ricken A, Eulitz D, Knoop H, Mannherz HG. Expression pattern of the deoxyribonuclease 1 gene: lessons from the Dnase1 knockout mouse. Biochem J. 2004 15;380(Pt 3):929-37.
17. Yusifov TN, Abduragimov AR, Gasymov OK, Glasgow BJ. Endonuclease activity in lipocalins. Biochem J. 2000;347:815-9.
18. Sakata M, Sack RA, Sathe S, Holden B, Beaton AR. Polymorphonuclear leukocyte cells and elastase in tears. Curr Eye Res. 1997;16:810-9.
19. Sonawane S, Khanolkar V, Namavari A, Chaudhary S, Gandhi S, Tibrewal S, Jassim SH, Shaheen B, Hallak J, Horner JH, Newcomb M, Sarkar J, Jain S. Ocular Surface Extracellular DNA and Nuclease Activity Imbalance: A New Paradigm for Inflammation in Dry Eye Disease. Invest Ophthalmol Vis Sci. 2012;53:8253-63.
20. McDermott AM. New insight into dry eye inflammation. Invest Ophthalmol Vis Sci. 2012;53:8264.

21. Hubbard RC, McElvaney NG, Birrer P, Shak S, Robinson WW, Jolley C, et al. A preliminary study of aerosolized recombinant human deoxyribonuclease I in the treatment of cystic fibrosis. *N Engl J Med* 1992;326:812–5.
22. Aitken ML, Burke W, McDonald G, Shak S, Montgomery AB, Smith A. Recombinant human DNase inhalation in normal subjects and patients with cystic fibrosis. *JAMA* 271 1992;267(14):1947–51.
23. Kalra L, Bone MF. The effect of nebulized bronchodilator therapy on intraocular pressures in patients with glaucoma. *Chest* 1988;93:739-41.
24. Geller DE. Clinical side effects during aerosol therapy: cutaneous and ocular effects. *J Aerosol Med.* 2007;20 Suppl 1:S100-8; discussion S109.
25. Harms HK, Matouk E, Tournier G, von der Hardt H, Weller PH, Romano L, Heijerman HG, FitzGerald MX, Richard D, Strandvik B, Kolbe J, Kraemer R, Michalsen H. Multicenter, open-label study of recombinant human DNase in cystic fibrosis patients with moderate lung disease. DNase International Study Group. *Pediatr Pulmonol.* 1998;26:155-61.
26. Hall SK: Acute angle-closure glaucoma as a complication of combined b-agonist and ipratropium bromide therapy in the emergency department. *Ann Emerg Med.* 1994;23:884-887.
27. Guner SN, Kilic M, Boyraz S, Yurdakul E, Kalkan G, Ozturk F. Bilateral posterior subcapsular cataracts after inhaled budesonide for asthma: have patients been given their medications correctly? *J Investig Allergol Clin Immunol.* 2011;21:80.
28. Weatherall M, Clay J, James K, Perrin K, Shirtcliffe P, Beasley R. *Respirology.* Dose-response relationship of inhaled corticosteroids and cataracts: a systematic review and meta-analysis. 2009;14:983-90.
29. Miller KL, Walt JG, Mink DR, Satram-Hoang S, Wilson SE, Perry HD, Asbell PA, Pflugfelder SC. Minimal clinically important difference for the ocular surface disease index. *Arch Ophthalmol.* 2010;128:94-101.
30. Lemp MA. Report of the National Eye Institute/Industry workshop on Clinical Trials in Dry Eyes. *CLAO J* 1995;21:221-32.
31. Schulze MM, Jones DA, Simpson TL. The development of validated bulbar redness grading scales. *Optom Vis Sci* 2007;84:976-83.
32. Amparo F, Dastjerdi MH, Okanobo A, Ferrari G, Smaga L, Hamrah P, Jurkunas U, Schaumberg DA, Dana R. Topical Interleukin 1 Receptor Antagonist for Treatment of Dry Eye Disease: A Randomized Clinical Trial. *JAMA Ophthalmol.* 2013;18:1-9.
33. Scuderi G, Contestabile MT, Gagliano C, Iacovello D, Scuderi L, Avitabile T. Effects of phytoestrogen supplementation in postmenopausal women with dry eye syndrome: a randomized clinical trial. *Can J Ophthalmol.* 2012;47:489-92.
34. Nichols JJ, Bickle KM, Zink RC, Schiwe MD, Haque RM, Nichols KK. Safety and efficacy of topical azithromycin ophthalmic solution 1.0% in the treatment of contact lens-related dry eye. *Eye Contact Lens* 2012;38:73-9.