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 Ocular Graft-*Vs.*-Host Disease

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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/ Transplant history	X							
Ophthalmic/ oGVHD history	X							
Vital signs		X		X	X	X	X	X
Best Spectacle Corrected Visual Acuity		X						X
Ocular Surface Disease Index (OSDI)	X	X		X	X	X	X	X
Clinical Global Impression (CGI)				X	X	X	X	X
Subject Global Assessment (SGA)				X	X	X	X	X
Visual Analogue Scale (VAS)			X	X	X	X	X	
Ophthalmic examination (Slit lamp)	X	X		X	X	X	X	X
Schirmer 1 test and Fluorescein and Lissamine Dye staining	X	X					X	
Ocular surface Redness (OR) score		X		X	X	X	X	X
Tear fluid analysis (PicoGreen, Cellometry)		X					X	
Keratograph Oculus Redness Score		X			X		X	
Non-invasive Keratography Tear Film Break-up Time (NIKBUT)		X			X		X	
Tear Meniscus Height (TMH)		X			X		X	
TearLab Osmolarity Test		X			X		X	
InflammaDry for MMP-9 Protein		X			X		X	
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Randomization visit		X						
First study medication		X						
Drug dispensation visits			X	X	X	X		
Instruction on study medication self-administration			X	X	X	X		

Physical inspection of nose & oropharynx		X		X	X	X	X	X
Adverse Events/ Concomitant Medications		X	X++	X	X	X	X	X
Conjunctival culture**		X					X	

***If applicable ++ AEs only**
****For select subjects only**

ABBREVIATIONS

ADR	Adverse Drug Reaction
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
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
NIKBUT	Non-invasive Keratograph Tear Film Break-Up Time
oGVHD	Ocular Graft-*Vs.*-Host Disease
OSDI	Ocular Surface Disease Index
Otc	Over the counter
QID	Four times per day
rhDNase I	Recombinant human deoxyribonuclease I
SAE	Serious Adverse Event
SGA	Subject Global Assessment
SP	Substance P
TMH	Tear Meniscus Height
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 ocular graft-*vs.*-host disease (oGVHD)
Short Title	RhDNase treatment for ocular GVHD
Protocol Number	UIC-CNBL-1002
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 rhDNase I eye drops in patients with ocular graft-vs.-host disease (oGVHD).
Number of Subjects	72 (study drug- 36, placebo- 36)
Diagnosis and Main Inclusion Criteria	Men and women ≥ 18 years of age with a diagnosis of oGVHD, with Schirmer I <10, Ocular surface staining ≥1 and OSDI mild (≥13).
Study Product, Dose, Route, Regimen	Study drug: rhDNase I (Pulmozyme®), 0.1% eye drops four times a day for eight weeks. Control: Vehicle eye drops four times a day for eight weeks.
Duration of administration	8 weeks
Reference therapy	Vehicle eye drops four times a day for eight weeks.
Statistical Methodology	One eye (target eye) will be selected at screening visit for statistical comparisons as follows: (i) if only 1 eye meets inclusion criteria, this eye is used; (ii) if both eyes meet inclusion criteria, the eye with the higher RBS score is used; (iii) if both eyes have the same RBS score, then the one with the lower Schirmer I score is used; (iv) if both eyes have same scores, the right eye is used. Secondary analyses will be performed for the non-target eye as well. To assess the effect of treatment, we will compare each outcome measure between rhDNase I (study) vs. Vehicle group over time using general linear mixed model, mixed-effects ordinal logistic regression, and mixed-effects multinomial logistic regression for continuous, ordinal and nominal variables respectively. Treatment, time and their interaction will be included in the model. Missing values of less than 25% will be imputed by using multiple imputations; otherwise will not be included in the analysis. Descriptive statistics will be utilized before the modeling to describe the sample. Assumptions behind each statistical method will be examined. Two-sided *P*-value less than 0.05 would be considered as statistical significance. All the analyses will be conducted by using SAS 9.2 (SAS Inst., Cary, NC).

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

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.

Ocular GVHD (oGVHD) and Dry Eyes: It is estimated that chronic GVHD affects 30–70% of allogeneic BMT recipients surviving beyond 100 days, with a median onset of 4–6 months following BMT.[1] Ocular manifestations, primarily tear-deficient dry eyes, are present in 60–90 % of patients with chronic GVHD.[2] The development of dry eyes has been attributed to causes beyond an allo-reactive immune reaction; for example, patients receiving autologous or syngeneic transplants who are consequently not at risk for the development of GVHD can also develop dry eyes.[3] oGVHD mimics other immunologically mediated inflammatory diseases of the ocular surface and there are no specific symptoms or clinical signs. oGVHD can affect quality of life by causing pain and photophobia, limiting activities of daily living (e.g., reading, watching television), compromising safety while driving, and permanently damaging vision.[3] Yet, in the absence of a complete understanding of the pathogenesis of chronic ocular GVHD, treatment remains empirical. Treatment options include lubricating eye drops, immunomodulator and steroid drops, and punctal occlusion. Relieving symptoms is difficult, and although multiple treatment options exist, many are ineffective.[3]

Although dry eye disease pathogenesis is not fully understood, inflammation has a prominent role in dry eyes symptom development and amplification.[4,5] 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.[6,7]

The immunopathological events that sustain the systemic adaptive immune response in dry eyes have been characterized using animal models of dry eye disease.[5-7] 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 dry eyes ocular surface inflammation and link the innate and adaptive immune mechanisms.

The ocular surface epithelium undergoes continuous, dynamic turnover,[8,9] which is increased in dry eyes patients.[10] Superficial corneal cells are shed into the precorneal tear film.[11,12] The corneal epithelial cell shedding process, or desquamation, is regulated by apoptotic mechanisms.[13] 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.[14,15] eDNA strands have been reported in corneal filaments, which are frequently present on the corneas of patients with severe dry eyes.[16] Desquamated cells in the precorneal tear film are a potential source of eDNA. Tear fluid nucleases, including lipocalin and DNase I, can hydrolyze and clear eDNA from the precorneal tear film.[17,18] Additionally, tear fluid contains several neutrophil extracellular trap (NET) components. Neutrophils undergo a low level of recruitment on the ocular surface,[19] and numerous neutrophils are present in the tear film during ocular surface inflammation.[20] Neutrophil elastase and histone proteins have also been reported in tear fluid.[21] 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.

1.2 Agent

The agent used in this study is recombinant human deoxyribonuclease I (rhDNase I). 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 (rhDNase I). The protein is produced by genetically engineered Chinese Hamster Ovary (CHO) cells containing DNA encoding for the native human protein, deoxyribonuclease I (DNase I). 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. Vehicle (placebo) contains all ingredients as Pulmozyme with the exception of the active ingredient (dornase alfa), and is dispensed in identical single-use ampules.

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 ≥ 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.[22,23] 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 ± SD) 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 have performed investigations to show that eDNA, NETs, and neutrophils are present on the ocular surface in oGVHD patients and abundant in mucoid films.[24,25] We showed that eDNA and NETs are present in excessive amounts on the ocular surface of patients with severe, tear-deficient dry eyes. Among all patients with dry eyes, those with GVHD had the highest eDNA amount in their tear fluid. 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 also significantly increased in dry eyes patients. Immunolocalization experiments revealed that the molecular components of NETs include histones, cathelicidin, and neutrophil elastase. Next, using a FRET-based assay, we found that tear fluid nuclease activity was reduced in dry eyes patients. We determined that the normal concentration of DNase I in tear fluid is 3.14 ng/ml, similar to that in serum and saliva.

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 dry eyes, 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. DNase I, an enzyme which selectively cleaves DNA, is one such therapeutic intervention. These findings have been published in Investigative Ophthalmology and Visual Sciences,[24,25] and were quoted as a new insight into dry eye inflammation by an editorial comment in the same publication.[26] Serum tear eye drops (containing DNase I) have been topically applied to the eye with significant therapeutic benefit to patients with severe dry eyes, including patients with oGVHD. These findings underpin the basis of this research protocol.

Our data reveals that patients with oGVHD are significantly more symptomatic with greater severity of clinical signs and higher tear fluid eDNA amount as compared to other tear-deficient dry eye disease subtypes,[25] therefore patients with oGVHD are ideal candidates for investigating clinical benefit that may result from clearing eDNA and NETs.

1.4 Clinical Data to Date

We have published case report of use of rhDNase I to treat a patient with severe, recalcitrant dry eyes due to ocular graft-*vs.*-host disease (oGVHD) who had excessive eDNA in their tear fluid.[25] rhDNase I (0.1%) eye drops were applied four times a day. 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 effects were observed (ocular inflammation or eye pressure changes). After 2 months of treatment with rhDNase I, corneal Rose Bengal staining and mucoid strands decreased and the patient reported increased ocular comfort. eDNA abundance in tear fluid was reduced and the pre-treatment growth in fluorescence signal reversed to a signal decay. Because eDNA is a possible source of inflammation in dry eyes,[24,26] reducing its abundance on the ocular surface with rhDNase I may have contributed to the observed clinical benefits. Furthermore, signal decay after rhDNase I treatment, as opposed to signal growth prior to treatment, suggests that the ocular surface cell turnover, which is increased in dry eyes, may have reverted to normal levels. Similar signal decay is seen in normal subjects. Thus, 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).

Additionally, 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.[27,28] 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.[29] 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).[30-32] Therefore, almost certainly, the eyes are exposed to study drug but there are no reports of serious adverse effects.

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 topical use of this drug.

1.5 Dose Rationale and Risk/Benefits

RhDNase I 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 rhDNase I topically. Since rhDNase I does not accumulate in tissues and its enzymatic activity is expected to be short-lived, we have elected to use the eye drops four times a day. The ocular residence time for emulsion eye drops (e.g. Restasis, used twice a day) has been estimated as approximately 2 hours.[33] We expect even shorter ocular residence time for rhDNase I, given the lower viscosity of its vehicle. Future clinical trials will be needed to assess whether lower frequency of dosing is also effective. The feasibility of performing this trial would have been suspect if we had included more than one dosing in this trial because of the much larger number of oGVHD patients needed.
Since rhDNase I 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 rhDNase I. Thus, we have chosen an eight weeks duration of therapy as it will give us better data to predict side effects during long-term use.

RhDNase I 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 using rhDNase I 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.

No single agent has yet been approved by the FDA for GVHD prevention or therapy.[34] Contact lenses, commonly used to treat oGVHD, will be withheld during the 14 day wash-out period and over the entire course of the study. Stopping contact lens wear may increase the subject's risk of worsening oGVHD. All study subjects will be encouraged to contact the research team immediately if they experience worsening of oGVHD.

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 ocular graft-vs.-host disease (oGVHD) are able to tolerate receiving rhDNase I 0.1% eye drops four times a day for eight weeks (primary tolerability objective) and to investigate the preliminary efficacy of rhDNase I 0.1% topical eye drop solution in treating oGVHD (primary efficacy objective).

3 Study Design

3.1 General Design

This will be a Randomized controlled trial, in which a total of 72 subjects will be enrolled at 1 clinical site. Subjects will be randomly assigned to one of two groups (#1, #2), with 36 subjects per group. One group will be given placebo (Vehicle eye drops) and the other group will be given eye drops containing the study drug (rhDNase I 0.1%).

Patients with established oGVHD will be approached by a member of the research staff to determine if he/ she might be interested in participating in a research study. If the subject is interested, the research staff member will describe the study. If the subject is willing to enter the study, the study will be discussed and the subject will be asked to sign the informed consent form. Consent will be obtained prior to screening to determine eligibility. Screening procedures include documentation of definite oGVHD. Eligible subjects 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.

Because NETs have an important role in the innate defense against microbes,[35] treatment with DNase I to clear NETs may increase susceptibility to ocular surface infections. Symptom relief is an important goal in patients having dry eyes, with the proviso that treatment does not interfere with pathogen defense. Therefore, we will determine whether DNase I treatment affects the ocular surface microbiome.

3.2 Primary Study Endpoints

Primary Efficacy End Point: The primary end point is a mean reduction in corneal staining score after 8 weeks of treatment, as measured by Fluorescein and Lissamine dye staining.

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

3.3 Secondary Study Endpoints

The secondary study endpoints will include:

1. The change in the Ocular Surface Disease Index (OSDI) which is a patient's subjective rating scale
2. Change in tear secretion as measured by Schirmer I test
3. The proportion of eyes achieving complete corneal staining clearance after treatment
4. Change in conjunctival surface staining score as measured by Fluorescein and Lissamine dye staining
5. The change in subjective ocular surface redness score (OR) using the validated bulbar redness (VBR) grading scale
6. Visual acuity change
7. Change in frequency of administration of artificial tears or concomitant eye drops
8. Change in number of corneal filaments (slit-lamp examination)
9. Change in amount of mucoid films (slit-lamp examination)
10. Clinical Global Impression (CGI) of change in symptoms from baseline (physician's rating)
11. Subject Global Assessment (SGA) of overall change from baseline (subject's rating)
12. Change in Non-Invasive Keratograph Tear Break-Up Time (NIKBUT)
13. Change in Tear Meniscus Height (TMH)
14. Change in Keratograph Ocular Bulbar Redness Score
15. Change in MMP-9 Protein Detection
16. Change in Tear Fluid Osmolarity
17. Change in eDNA abundance in tear fluid
18. Change in tear fluid cell count
19. Change in eDNA strand length on Schirmer test strip impressions

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

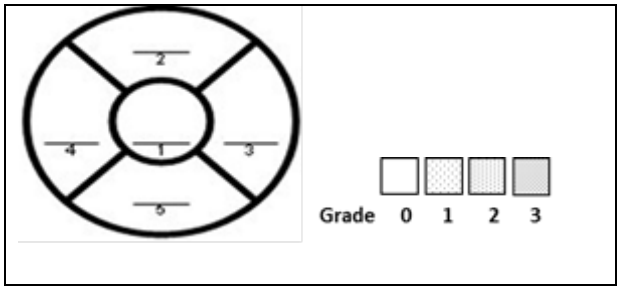
3.3.1 Efficacy End Point: Ocular surface Fluorescein and Lissamine Dye staining score

Ocular surface staining will be assessed using Fluorescein dye for Cornea and Lissamine Green Dye for conjunctiva. The scoring pattern is represented below.

Fluorescein Dye for Cornea:

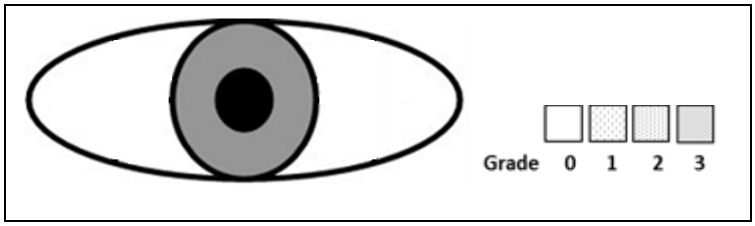
Using the insulin syringe, 2 units (20uL) of the 1ml insulin syringe will be drawn out from the fluorescein bottle and fluorescein in the syringe will be released into sterile 1ml Eppendorf vial and capped. Using 5µL fixed pipette and a 10µL pipette tip 5µL of the fluorescein will be drawn from the Eppendorf vial and after slightly pulling down the right lower eye lid of the patient fluorescein will be released into the lower conjunctival sac. After waiting for 30 seconds the process will be repeated in the left eye. Recording of corneal fluorescein staining will be performed by a slit lamp examination after 2 minutes and 30

seconds for each eye using the grading system described below. Corneal staining will be graded in 5 zones
Each zone will be graded from 0 to 3 based on the density of punctate staining.



Lissamine Green Dye for Conjunctiva:

Using the insulin syringe, 2 units (20uL) of the 1ml insulin syringe will be drawn out from the Lissamine bottle and Lissamine in the syringe will be released into sterile 1ml Eppendorf vial and capped. Using 5uL fixed pipette and a 10uL pipette tip 5uL of the Lissamine will be drawn from the Eppendorf vial and after slightly pulling down the right lower eye lid of the patient Lissamine will be released into the lower conjunctival sac. After waiting for 30 seconds the process will be repeated in the left eye. Using the slit lamp (white light of moderate intensity) nasal-bulbar and temporal-bulbar conjunctiva will be graded within 2 minutes for each eye.



3.3.2 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:[37]

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

3.3.3 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 subjects' change in symptoms and signs. The CGI is a follows:[38]

Question (to physician): In general, compared with the subjects' 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

3.3.4 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:[38]

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

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

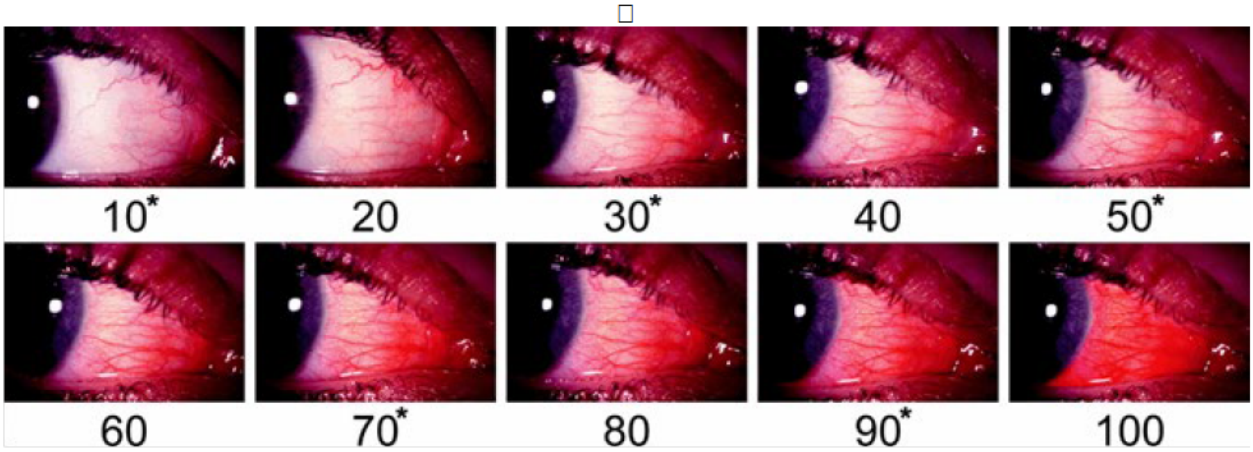
Much worse
Worse
About the same
Improved
Much improved

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

3.3.5 Efficacy End Point: Ocular surface redness score

Ocular surface redness (nasal or temporal) will be assessed using the Validated Bulbar Redness grading scale (VBR).[39] 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 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:



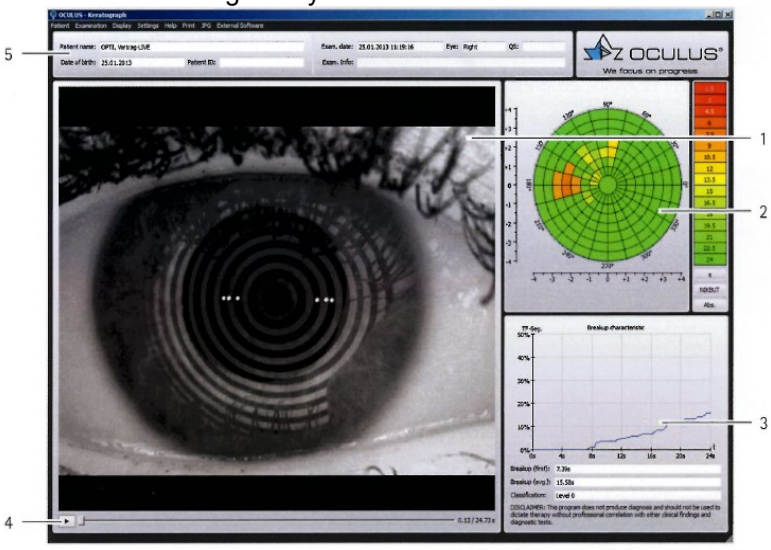
3.3.6 Efficacy End Point: Keratograph Oculus Redness Score

Keratograph is FDA approved and is used in routine clinical care of patients in the US. The Oculus Keratograph 5M performs a non-invasive tear film analysis. It uses a Placido bowl with a camera aperture that has a fixation mark in the center. The device provides consistent illumination, allowing scanning of the exposed bulbar conjunctiva to take place. The keratograph then analyzes the scanned area. This system generates a BR score automatically, which is based on the area percentage ratio between the vessels and the rest of the analyzed area.

For instance, if the ratio is 16%, then the score is 1.6. The maximum ratio, according to the manufacturer, is 40%; therefore, the BR scores that the machine generates range between 0.0 and 4.0.

Non-invasive Keratograph Tear Film Break-up Time (NIKBUT)

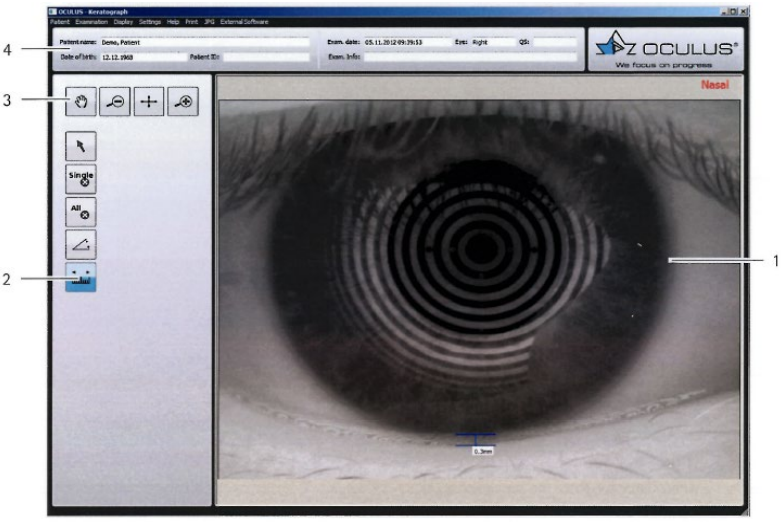
The non-invasive Keratograph tear film break-up time (NIKBUT) measures tear film stability. The NIKBUT is automatically measured within seconds, without fluorescein application. Tear Break-up Time (TBUT) will be measured twice for each eye using IR video derived from the Oculus noninvasive Keratograph tear breakup time (NIKBUT) tool. Based on the device IR video, the device generates 2 measures for TBUT: NIKBUT-first (time at which the first breakup of tears occurs) and NIKBUT-average (average time of all breakup incidents) automatically and without touching the eye.



1 *Camera image*
2 *Color map of the tear film break-up time*
3 *Graphic display of the tear film break-up time characteristics*
4 *Start-stop function for the video with time display*
5 *Examination and patient data*

Tear Meniscus Height (TMH)

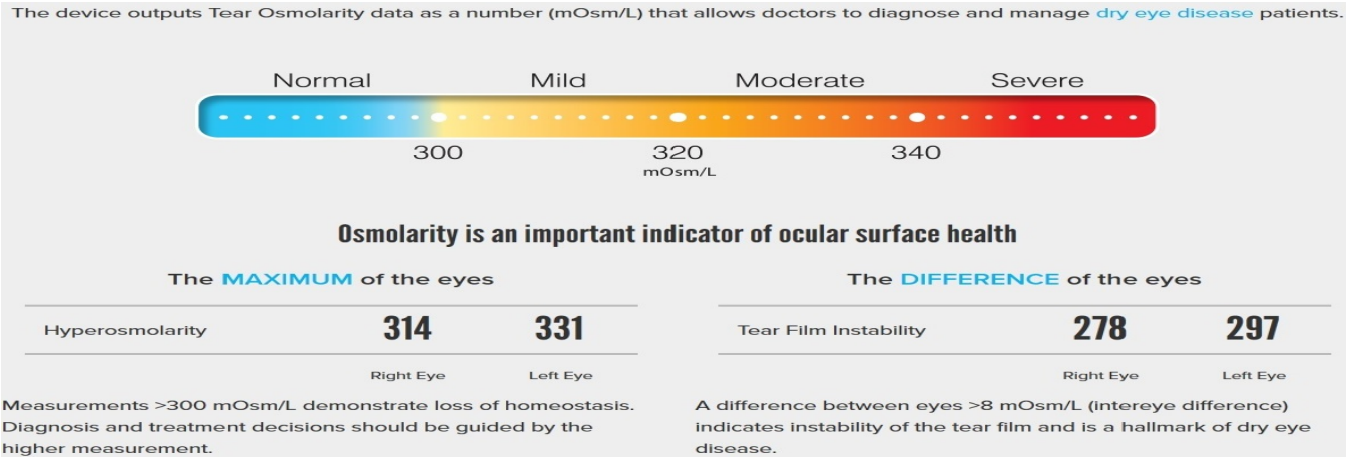
Tear Meniscus Height (TMH) Measurement evaluates the course of the tear meniscus along the eyelid by means of the new infrared illumination and precisely measure the tear meniscus height with an integrated ruler. The TMH is measured twice for each eye using IR images derived from the Oculus TMH tool, The TMH will be graded perpendicular to the lid margin at the central point relative to the pupil center. Oculus TMH measurement is generated automatically by Oculus K5M software. The tear meniscus measurement is important for determining the tear film quantity.



1 *Camera image*
2 *Buttons for tear meniscus measurement*
3 *Buttons to work in the camera image*
4 *Examination and patient data*

3.3.7 Efficacy End Point: TearLab Osmolarity Test

TearLab Osmolarity Test is FDA approved and is used in routine clinical care of patients in the US. The TearLab Osmolarity Test is intended to measure the osmolarity of human tears to aid in the diagnosis of dry eye disease in patients suspected of having dry eye disease. Abnormal tear osmolarity is a failure of homeostatic osmolarity regulation, a key feature of dry eye disease (DED). When left unchecked, hyperosmolar tears in early stage DED will lead to damage of the cornea and conjunctiva evident in later stage disease. The higher the osmolality, the more severe the dry eye. The TearLab Osmolarity Test provides a quick and simple method for determining tear osmolarity using nanoliter (nL) volumes of tear fluid collected directly from the eyelid margin. The TearLab Osmolarity Test utilizes a temperature-corrected impedance measurement to provide an indirect assessment of osmolarity. After applying a lot-specific calibration curve, osmolarity is calculated and displayed as a quantitative numerical value.



3.3.8 Efficacy End Point: InflammaDry for MMP-9 Protein

InflammaDry is FDA approved and is used in routine clinical care of patients in the US. Elevated levels of the MMP-9 protein in human tears will be detected visually, qualitatively, and in vitro using InflammaDry for MMP-9. InflammaDry will be performed PRIOR to instilling ocular anesthetic, topical dyes, or performing Schirmer testing. Tear fluid sample will be collected by gently dabbing the sampling fleece on the inside of the patient's palpebral conjunctiva a minimum of six to eight (6-8) dabs along the conjunctiva, afterwards allow the sampling fleece to rest against the conjunctiva for an additional five (5) seconds. Assemble the test by gently placing the sampling fleece of the sample collector into the sample transfer window of the test cassette body. Open the buffer vial and immerse the absorbent tip for a minimum of 20 seconds. Remove the absorbent tip from the buffer vial, replace the protective cap, and lay the test flat on a horizontal surface for ten (10) minutes.

The results of the test are indicated through two (2) lines, the control line and the result line in the result window.

POSITIVE RESULT: The presence of both a BLUE line in the control zone and a RED line in the result zone indicates a positive result. A positive result indicates the presence of MMP-9 >= 40 ng/ml.
NEGATIVE RESULT: The presence of only a BLUE line in the control zone indicates a negative result. A negative result is indicative of an MMP-9 < 40 ng/ml.
INVALID RESULT: If a BLUE line does not appear, the test may be invalid. Re-immerse the absorbent tip into the buffer vial for an additional ten (10) seconds. If a BLUE line still does not appear, the test must be discarded and the subject retested by resampling the eye using a new InflammaDry test.

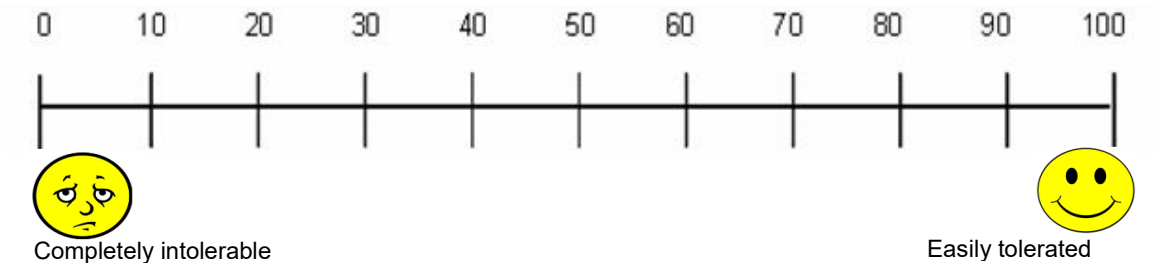
3.3.9 Tolerability End Point: Test Substance Tolerance (Visual Analogue Scale)

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.



3.4 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 (^{0}F). Subjects with an oral temperature less than (≤) 99.6^{0}F (*37.4^{0}C*) 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. Also, conjunctival and eyelid margin swabs will be taken for microbiologic cultures at baseline and at week 8 to assess potential changes in bacterial flora in a subset of participants (20 subjects each, in study and control groups). Because of the masking, selection of subjects for conjunctival cultures will be made by the pharmacist incharge for randomization.

3.4.3 Other Study procedures

At the first and last treatment visits, subjects will be asked to allow sampling of their tear fluid (6-10 µl) for laboratory assays. Tears will be collected from the lower lid tear meniscus and inferior fornix using a blunt glass microcapillary tube. In subjects with severe tear deficiency, a drop of preservative-free artificial tears will be instilled in the eye and conjunctival washings will be collected after a 2-minute period. Assays for eDNA abundance will be performed in PI's laboratory using the Quant-iT™ PicoGreen® dsDNA Assay Kit as we described previously.[25] Number of cells in tear fluid will be counted using an automated cell counter.

Also, Schirmer test strips that are discarded after the Schirmer I test will be used to make impressions on glass slides and immunofluorescence staining will be performed in PI's laboratory.[24] Five random 20x fields will be imaged using an inverted microscope and analyzed. eDNA strands will be traced and lengths calculated using Neurolucida software.

4 Subject Selection and Withdrawal

4.1 Inclusion Criteria

The PI and other members of International Chronic Ocular GVHD Consensus Group have established the consensus diagnostic criteria and classification for chronic ocular GVHD.[40]

Table 1: Severity scale in chronic ocular GVHD

Severity scores (points)	Schirmer's test (mm)	CFS (points)	OSDI (points)	Conj (points)
0	>15	0	<13	None
1	11–15	<2	13–22	Mild/Moderate

Severity scores (points)	Schirmer's test (mm)	CFS (points)	OSDI (points)	Conj (points)
2	6–10	2–3	23–32	Severe
3	≤5	≥4	≥33	

CFS; corneal fluorescein staining, OSDI; Ocular Surface Disease Index. Conj; conjunctival injection. Severity classification; Total score (points); (Schirmer's test score+ CFS score+ OSDI score+ Conj injection score) = None;0–4, Mild/Moderate; 5–8, Severe, 9–11.

Table 2: Diagnosis of chronic ocular GVHD

	None (points)	Probable GVHD (points)	Definite GVHD (points)
Systemic GVHD(−)	0–5	6–7	≥8
Systemic GVHD(+)	0–3	4–5	≥6

Based on these criteria (Tables 1 and 2),[40] patients with definite ocular GVHD will be enrolled. Additionally, all of the following criteria should be met to be eligible for the study:

- Aged 18 years or older.
- Capable of giving informed consent and does provide informed consent.
- Schirmer I <10
- Corneal/ conjunctival (Fluorescein and Lissamine Dye) staining ≥1
- Ocular symptoms must be considered as annoying or activity limiting (OSDI ≥13; mild).
- 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:

- Allergic to rhDNase I or any similar products, or excipients of rhDNase I eye drops 0.1%.
- Receiving or have received within 30 days any experimental systemic medication.
- Active ocular infection or ocular allergies.
- Any history of eyelid surgery or ocular surgery within the past 3 months.
- Corneal epithelial defect larger than 1 mm^2 in either eye.
-
- Have active drug/alcohol dependence or abuse history.
- Vulnerable populations, such as neonates, pregnant women, children, prisoners, institutionalized individuals, or others who may be considered vulnerable populations.

Participants will be permitted to continue their chronic GVHD treatments, including the use of artificial tears, eyelid massage, or warm compresses. Subjects wearing contact lenses (Soft bandage contact lenses or PROSE lens) will be asked to discontinue contact lens wear for 1 month prior to being

enrolled (Baseline visit) and will be required to not wear contact lenses for the duration of the study (until week 10 visit).

4.3 Subject Recruitment and Screening

Potential subjects will be recruited from the clinical practice of the investigator 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 (UIC). Subjects will include patients who have been diagnosed with oGVHD in the investigator's eye clinic (cornea clinic or the comprehensive eye clinic), in the Illinois Eye and Ear Infirmary. Patients diagnosed with oGVHD, and willing to enter the study, may be referred to the PI's clinic by other ophthalmologists at UIC, as well as the ophthalmology departments at Loyola University Chicago, University of Chicago, and Northwestern University. These centers will be provided with 'information sheets' to provide to subjects before they come to the PI's clinic. Although patients may be referred from these sites, no study procedures, including enrollment will be performed at any site, other than the PI's clinic in Illinois Eye and Ear Infirmary. All subjects will be will be screened, recruited, and will attend all study related visits only at the PI's clinic in Illinois Eye and Ear Infirmary.

Patients with definite ocular GVHD (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 subject might be interested in participating in a research study. If the subject is interested, the research staff member will describe the study. If the subject is willing to enter the study, the subject will be asked to review the study consent form, the PI will meet with the subject to review the form, the study will be discussed to confirm the subject's understanding of the study, and to answer any questions that the subject might have. Once the subject demonstrates understanding of the study and agrees to participate in the study, the subject will be asked to sign the informed consent form in the presence of the PI. Consent will be obtained prior to screening to determine eligibility. Screening procedures include documentation of definite oGVHD as well as other assessments as detailed in section 6.5.1. Eligible subjects 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, rhDNase I, will be supplied as a 1 mg/ml (0.1%) solution for administration as topical eye drops. The control group will receive Vehicle eye drops as placebo. Vehicle (placebo) contains all ingredients as Pulmozyme with the exception of the active ingredient (dornase alpha), and is dispensed in identical single-use ampules.

Each subject will receive either 0.1% rhDNase I (study drug) or 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- rhDNase I, 1mg/ml (0.1%) eye drops will be applied to both eyes q.i.d for 8 weeks. Control group – Vehicle eye drops will be applied to both eyes q.i.d for 8 weeks. For either group, the subject 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). One group will receive the study drug (rhDNase 0.1%; test group), and the other group will receive placebo (Vehicle; control 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/ #2). 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 or study drug. 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 subject 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. No modifications will be made to the study medication constituents. The drug will be re-packaged in sterile eye droppers for dispensing, as explained below:
The study medications will be dispensed in sterile eye droppers of 3 ml volume. The commercially available product is a single-use ampule containing 2.5 mL of study drug. The medication will be transferred to two sterile eye droppers of 3 ml volume. Approximately 1 ml of study drug will be transferred to each sterile eye dropper, thus 2 eye droppers (each containing 1 ml of the study medications) will be made from each commercially available ampule (containing 2.5 mL of study drug). This repackaging will be done at Illinois eye and ear infirmary (EEI) pharmacy, under standard aseptic precautions. The eye
droppers will be used by subjects 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 ^{0}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 subject.

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 subject's medical record, including all lubricants administered for oGVHD. These medications will be recorded in the case report form (CRF).

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

- Medication trade name
- Eye that was treated, if applicable
- Indication for which the medication was given
- Date started
- Date stopped
- Dose of medication used.

In general, patients will be required to maintain the treatments that they were using at entry into the study throughout the follow-up period. Patients will be instructed to continue using the same brand of eye drops during the study as they were using at the screening visit. If patients are wearing contact lenses (Soft bandage contact lenses or PROSE lens), they will be asked to discontinue contact lens wear for 1 month prior to being enrolled (Baseline visit) and will be required to not wear contact lenses for the duration of the study (until week 10 visit). Patients will be enrolled if they have been using their eye treatments for at least 30 day prior to the baseline visit and patients will be asked to commit to continue using the same treatments for the duration of the study. Participants will be also be permitted to continue ancillary treatments such as eyelid massage or warm compresses of the eyelids. The number of drops and frequency must be recorded in the subject diary provided.

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

<u>There is currently no FDA approved treatment available to treat patients with oGVHD</u>

Contact lenses, commonly used to treat oGVHD, will be withheld during the course of this study. This is necessary as the proposed primary efficacy outcome measure is corneal Fluorescein and Lissamine Dye Staining. Contact lenses can directly reduce corneal SPK and staining due to ensconcing and acting like a bandage that prevents corneal exposure to tear fluid inflammatory materials. Thus, because of over lapping effects on the primary outcome measure, it may not be possible to attribute any observed clinical benefit to rhDNase I treatment if contact lenses are 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 (NIH funded 'Dry Eye Evaluation and Management (DREAM) study').[41]

Stopping contact lens wear may increase the risk of worsening of oGVHD during the wash-out period and during the course of the study. However, because other eye drop treatments will continue during

the wash out period as well as during the course of the study, 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 oGVHD, 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 oGVHD 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) Increasing 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 contact lens wear is instituted during the course of the study and/or the study drug is withdrawn, the subject will be withdrawn 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 oGVHD 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) Increasing 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 contact lens wear is reinstituted 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 subject 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 Subject Recruitment and Screening

Prior to recruitment of any subjects 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 routine 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 oGVHD will be approached by a member of the research staff to determine if the subject might be interested in participating in a research study. If the subject is interested, the research staff member will describe the study. If the subject is willing to enter the study, the subject will be asked to review the

study consent form, the PI will meet with the subject to review the form, the study will be discussed to conform the subject's understanding of the study, and to answer any questions that the subject might have. Once the subject demonstrates understanding of the study and agrees to participate in the study, the subject will be asked to sign the informed consent form in the presence of the PI. Patients that contact the researchers in response to a flyer and information sheet provided by their physician will be scheduled for a visit to discuss the study and participate in the consent process. 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 subjects will be enrolled in the study.

6.2 Assignment of Subject 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 Subject Enrollment

Subjects 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 history of systemic (acute or chronic) GVHD, stem cell transplant history (including the date and type of transplant, degree of mismatch, if any, etc.), 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 history including: date when the oGVHD began, verification that the subject has had definite oGVHD, medications used by the subject to treat oGVHD, previous procedures to treat oGVHD. Ocular symptoms due to oGVHD will be quantified using the Ocular Surface Disease Index (OSDI) questionnaire (section 3.3.2).
- Ophthalmic Examination (slit lamp examination, Ocular Surface Redness Score, Fluorescein and Lissamine Dye 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.

Subjects currently treating oGVHD with corticosteroids and/or Restasis will continue these treatments during the whole study. If patients are wearing contact lenses (Soft bandage contact lenses or PROSE lens), they will be asked to discontinue contact lens wear for 1 month prior to being enrolled (Baseline visit) and will be required to not wear contact lenses for the duration of the study (until week 10 visit). The study doctor will provide advice about decreasing oGVHD symptoms with artificial tears, eyelid massage, or warm compresses.

If the subject 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 (±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).
- BSCVA (Snellen's chart)
- OSDI
- Schirmer 1 test and Fluorescein and Lissamine Dye staining
- Keratograph Oculus Redness Score
  > Non-invasive Keratography Tear Film Break-up Time (NIKBUT)
  > Tear Meniscus Height (TMH)
- TearLab Osmolarity Test
- InflammaDry Test for MMP-9 Protein
- Baseline Ophthalmic Examination (slit lamp examination, Ocular surface redness score)
- Tear fluid collection for analysis (PicoGreen, Cellometry)
- Physical Inspection of the Nose and Oropharynx
- Conjunctival and eyelid margin cultures
- Record changes in concomitant medication
- Adverse events since screening visit.

Randomization (by pharmacy)

Investigator/designee administers first dose

Post-Dose

- Test Substance Tolerance (Visual Analogue Scale; VAS)
- 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
- OSDI
- Clinical Global Impression, Subject Global Assessment
- Test Substance Tolerance (VAS)
- Ophthalmic Examination (slit lamp examination, Ocular surface redness score)
- Physical Inspection of the Nose and Oropharynx
- 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
- OSDI
- Keratograph Oculus Redness Score
  > Non-invasive Keratography Tear Film Break-up Time (NIKBUT)
  > Tear Meniscus Height (TMH)
- TearLab Osmolarity Test
- InflammaDry Test for MMP-9 Protein
- Clinical Global Impression, Subject Global Assessment
- Test Substance Tolerance (VAS)
- Ophthalmic Examination (slit lamp examination, Ocular surface redness score)
- Physical Inspection of the Nose and Oropharynx
- 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 (± 2 days)*

Treatment Visit (after any dose for that day)

- Vital Signs
- OSDI
- Clinical Global Impression, Subject Global Assessment
- Test Substance Tolerance (VAS)
- Ophthalmic Examination (slit lamp examination, Ocular surface redness score)
- Physical Inspection of the Nose and Oropharynx

- 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 (± 2 days)*

Treatment Visit (after any dose for that day)

- Vital Signs
- OSDI
- Fluorescein and Lissamine Dye staining, and Schirmer 1 test
- Keratograph Oculus Redness Score
  > Non-invasive Keratography Tear Film Break-up Time (NIKBUT)
  > Tear Meniscus Height (TMH)
- TearLab Osmolarity Test
- InflammaDry Test for MMP-9 Protein
- Clinical Global Impression, Subject Global Assessment
- Test Substance Tolerance (VAS)
- Ophthalmic Examination (slit lamp examination, Ocular surface redness score)
- Tear fluid collection for analysis (PicoGreen, Cellometry)
- Physical Inspection of the Nose and Oropharynx
- Conjunctival and eyelid margin cultures
- 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 (± 2 days)

Follow-Up Visit

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

7 Statistical Plan

7.1 Sample Size Determination

The sample size is based on the primary efficacy end point (corneal RBS score) using the following assumptions: **(i)** mean RBS score in ocular GVHD population = 3.1, **(ii)** sigma (standard deviation) = 2.5, **(iii)** expected mean based on 60% effect = 1.24. These assumptions are based on our preliminary data in ocular GVHD patients where we found that treatment with DNase I eye drops reduced RBS score by 80% (mean).[25] A total of 30 subjects in each group will be required to detect the difference between groups using a 2-sided two-sample *t* test with an alpha level of 0.05 and 80% power. We elected to enroll 36 subjects per group.

7.2 Statistical Methods

Biostatisticians from the Design and Analysis Core (DAC) of UIC-CCTS will perform the analysis. We will assess treatment efficacy based on the intent-to-treat principle using each participant's randomized treatment assignment to define treatment groups and outcomes. Safety variables will be analyzed with regard to treatment that participants actually received (the safety population). One eye (target eye) will be selected at screening visit for statistical comparisons as follows: **(i)** if only 1 eye meets inclusion criteria, this eye is used; **(ii)** if both eyes meet inclusion criteria, the eye with the higher RBS score is used; **(iii)** if both eyes have the same RBS score, then the one with the lower Schirmer I score is used; **(iv)** if both eyes have same scores, the right eye is used. Secondary analyses will be performed for the non-target eye as well. To assess the effect of treatment, we will compare each outcome measure between rhDNase I (study) vs. Vehicle (control) group over time using general linear mixed model, mixed-effects ordinal logistic regression, and mixed-effects multinomial logistic regression for continuous, ordinal and nominal variables respectively. Treatment, time and their interaction will be included in the model. Missing values of less than 25% will be imputed by using multiple imputations; otherwise will not be included in the analysis. Descriptive statistics will be utilized before the modeling to describe the sample. Assumptions behind each statistical method will be examined. Two-sided *P*-value less than 0.05 would be considered as statistical significance. All the analyses will be conducted by using SAS 9.2 (SAS Inst., Cary, NC).

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 AE is any unfavorable and unintended sign, symptom, or disease temporally associated with the use of an investigational medicinal product (IMP) or other protocol-imposed intervention, regardless of attribution. This includes the following:

- AEs not previously observed in the subject that emerge during the protocol-specified AE reporting period, including signs or symptoms associated with ocular-graft-vs.-host disease (oGVHD) that were not present prior to the AE reporting period.
- Complications that occur as a result of protocol-mandated interventions.
- If applicable, AEs that occur prior to assignment of study treatment associated with medication washout, no treatment run-in, or other protocol-mandated intervention.
- Preexisting medical conditions (other than the condition being studied) judged by the investigator to have worsened in severity or frequency or changed in character during the protocol-specified AE reporting period

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.

All AEs and SAEs whether volunteered by the subject, discovered by study personnel during questioning, or detected through physical examination, laboratory test, or other means will be reported appropriately. 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 AE should be classified as an SAE if the following criteria are met:

1. It results in death (i.e., the AE actually causes or leads to death.).
2. It is life-threatening (i.e., the AE, in the view of the investigator, places the subject at immediate risk of death. It does not include an AE that, had it occurred in a more severe form, might have caused death.).
3. It requires or prolongs inpatient hospitalization.
4. It results in persistent or significant disability/incapacity (i.e., the AE results in substantial disruption of the subject's ability to conduct normal life functions).
5. It results in a congenital anomaly/ birth defect in a neonate/infant born to a mother exposed to the investigational medicinal product (IMP).
6. It is considered a significant medical event by the investigator based on medical judgement (e.g., my jeopardize the subject or may requires medical or surgical intervention to prevent any of the occurrences listed above.

To ensure consistency of AE and SAE causality assessments, the following general guideline will be applied:

Yes
There is a plausible temporal relationship between the onset of the AE and administration of the Pulmozyme, and the AE cannot be readily explained by the subject's clinical state, intercurrent illness, or concomitant therapies; and/or the AE follows a known pattern of response to the Pulmozyme; and/or the AE abates or resolves upon discontinuation of the Pulmozyme or dose reduction and, if applicable, reappears upon re-challenge

No
Evidence exists that the AE has an etiology other than the Pulmozyme (e.g., preexisting medical condition, underlying disease, intercurrent illness, or concomitant medication); and/or the AE has no plausible temporal relationship to Pulmozyme administration (e.g., cancer diagnosed 2 days after first dose of study drug)

• ***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.
Expected adverse event: Expected adverse events are those adverse events that are listed or characterized in the Package Insert (P.I) or current Investigator Brochure (I.B).
• ***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 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

8.4 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.

8.5 PROCEDURES FOR ELICITING, RECORDING, AND REPORTING ADVERSE EVENTS

8.5.1 Eliciting Adverse Events

A consistent methodology for eliciting AEs at all subject evaluation time points will be adopted. Examples of non-directive questions include:

- "How have you felt since your last clinical visit?"
- "Have you had any new or changed health problems since you were last here?"

8.5.2 Specific Instructions for Recording Adverse Events

Investigators should use correct medical terminology/concepts when reporting AEs or SAEs. Avoid colloquialisms and abbreviations.

a. Diagnosis vs. Signs and Symptoms

If known at the time of reporting, a diagnosis should be reported rather than individual signs and symptoms (e.g., record only liver failure or hepatitis rather than jaundice, asterixis, and elevated transaminases). However, if a constellation of signs and/or symptoms cannot be medically characterized as a single diagnosis or syndrome at the time of reporting, it is acceptable to report the information that is currently available. If a diagnosis is subsequently established, it should be reported as follow-up information.

b. Deaths

All deaths that occur during the protocol-specified AE reporting period (see Section I), regardless of attribution, will be reported to the appropriate parties. When recording a death, the event or condition that caused or contributed to the fatal outcome will be reported as the single medical concept. If the cause of death is unknown and cannot be ascertained at the time of reporting, report “Unexplained Death”.

c. Preexisting Medical Conditions

A preexisting medical condition is one that is present at the start of the study. Such conditions will be reported as medical and surgical history. A preexisting medical condition should be re-assessed throughout the trial and reported as an AE or SAE only if the frequency, severity, or character of the condition worsens during the study. When reporting such events, it is important to convey the concept that the preexisting condition has changed by including applicable descriptors (e.g., “more frequent headaches”).

d. Hospitalizations for Medical or Surgical Procedures

Any AE that results in hospitalization or prolonged hospitalization should be documented and reported as an SAE. If a subject is hospitalized to undergo a medical or surgical procedure as a result of an AE, the event responsible for the procedure, not the procedure itself, should be reported as the SAE. For example, if a subject is hospitalized to undergo coronary bypass surgery, record the heart condition that necessitated the bypass as the SAE.

Hospitalizations for the following reasons do not require reporting:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for preexisting conditions
- Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study or
- Hospitalization or prolonged hospitalization for scheduled therapy of the target disease of the study.

e. Pregnancy

If a female subject becomes pregnant while receiving the study drug or within 30 days after the last dose of study drug, a report will be completed and expeditiously submitted to Genentech, Inc. Follow-up to obtain the outcome of the pregnancy will also occur. Abortion, whether accidental, therapeutic, or spontaneous, should always be classified as serious, and expeditiously reported as an SAE. Similarly, any congenital anomaly/birth defect in a child born to a female subject exposed to the {study drug} should be reported as an SAE.
Additional information on any Pulmozyme (rhDNase)-exposed pregnancy and infant will be requested by Roche Drug Safety at specific time points (i.e. after having received the initial report, at the end of the second trimester, 2 weeks after the expected date of delivery, and at 3, 6, and 12 months of the infant’s life).

f. Post-Study Adverse Events

The investigator should expeditiously report any SAE occurring after a subject has completed or discontinued study participation if attributed to prior {study drug} exposure. If the investigator should become aware of the development of cancer or a congenital anomaly in a subsequently conceived offspring of a female subject who participated in the study, this should be reported as an SAE.

g. Reconciliation

The Sponsor agrees to conduct reconciliation for the product. Genentech and the Sponsor will agree to the reconciliation periodicity and format, but agree at minimum to exchange quarterly (no less frequently than monthly) line listings of cases received by the other party. If discrepancies are identified, the Sponsor and Genentech will cooperate in resolving the discrepancies. The responsible individuals for each party shall handle the matter on a case-by-case basis until satisfactory resolution. The sponsor shall receive reconciliation guidance documents within the 'Activation Package'.

8.5.3 Adverse Event Reporting

All subjects who have been exposed to study drug will be evaluated for adverse events. The study period during which all AEs and SAEs will be reported begins after informed consent is obtained and initiation of study treatment and ends 21 days following the last administration of study treatment or study discontinuation/termination, whichever is earlier. AE reports and annual summaries will not include subject identifiable material. Each report will only include the identification code. 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.

8.6 Adverse Event Reporting

8.6.1 AEs of Special Interest (AESIs)

AEs of Special Interest are defined as a potential safety problem, identified as a result of safety monitoring of the Product. There are no AESIs for pulmozyme.

8.6.2 Adverse Event Reporting

Investigator will report all SAEs to FDA, IRB, NEI, and Genentech within the timelines described below. The completed MedWatch/case report will be faxed immediately upon completion to Genentech Drug Safety at:

(650) 225-4682
OR
(650) 225-4630

Serious adverse events (SAEs), pregnancy reports and AEs of special interest (AESIs), where the patient has been exposed to the Product, will be sent on a MedWatch or CIOMS I form to the Roche contact. Transmission of these reports (initial and follow-up) will be either electronically or by fax and within the timelines specified below:

- **SADRs**

Serious AE reports that are related to the Product shall be transmitted to Roche within fifteen (15) calendar days of the awareness date.

- **Other SAEs**

Serious AE reports that are unrelated to the Product shall be transmitted to Roche within thirty (30) calendar days of the awareness date.

- **Pregnancy reports**

While such reports are not serious AEs or ADRs per se, as defined herein, any reports of pregnancy, where the fetus may have been exposed to the Product, shall be transmitted to Roche within thirty (30) calendar days of the awareness date. Pregnancies will be followed up until the outcome of the pregnancy is known, whenever possible, based upon due diligence taken to obtain the follow-up information.

Special situation reports

In addition to all AEs, pregnancy reports and AESIs, the following Special Situations Reports should be collected and transmitted to Roche even in the absence of an Adverse Event within thirty (30) calendar days:

- Data related to the Product usage during pregnancy or breastfeeding
- Data related to overdose, abuse, off-label use, misuse, inadvertent/erroneous administration, medication error or occupational exposure, with or without association with an AE/SAE unless otherwise specified in the protocol
- Data related to a suspected transmission of an infectious agent via a medicinal product (STIAMP)
- Lack of therapeutic efficacy

In addition, reasonable attempts will be made to obtain and submit the age or age group of the patient, in order to be able to identify potential safety signals specific to a particular population.

Aggregate Reports

Dr. Sandeep Jain will forward a copy of the Publication to Roche upon completion of the Study.

8.6.3 MedWatch 3500A Reporting Guidelines

In addition to completing appropriate patient demographic and suspect medication information, the report should include the following information within the Event Description (section 5) of the MedWatch 3500A form*:*

- Protocol description (and number, if assigned)
- Description of event, severity, treatment, and outcome if known
- Supportive laboratory results and diagnostics
- Investigator's assessment of the relationship of the adverse event to each investigational product and suspect medication

8.6.3.1 Follow-up Information

Additional information may be added to a previously submitted report by any of the following methods:

- Adding to the original MedWatch 3500A report and submitting it as follow-up
- Adding supplemental summary information and submitting it as follow-up with the original MedWatch 3500A form
- Summarizing new information and faxing it with a cover letter including patient identifiers (i.e. D.O.B. initial, patient number), protocol description and number, if assigned, brief adverse event description, and notation that additional or follow-up information is being submitted (The patient identifiers are important so that the new information is added to the correct initial report)

Occasionally Genentech may contact the reporter for additional information, clarification, or current status of the patient for whom an adverse event was reported. For questions regarding SAE reporting, you may contact the Genentech Drug Safety representative noted above or the MSL assigned to the study. Relevant follow-up information should be submitted to Genentech Drug Safety as soon as it becomes available and/or upon request.

MedWatch 3500A (Mandatory Reporting) form is available at
http://www.fda.gov/medwatch/getforms.html

8.7 Study Close-Out

Any study report submitted to the FDA by the Sponsor-Investigator should be copied to Genentech. This includes all IND annual reports and the Clinical Study Report (final study report). Additionally, any literature articles that are a result of the study will be sent to Genentech. Copies of such reports will be mailed to the assigned Clinical Operations contact for the study:

pulmozyme-gsur@gene.com

8.7.1: Additional Reporting Requirements for IND Holder

For Investigator-Initiated IND Studies, events meeting the following criteria will be submitted to the Food and Drug Administration (FDA) as expedited IND Safety Reports according to the following guidance and timelines:

8.7.1.1 7 Calendar Day Telephone or Fax Report

The Investigator will notify the FDA of any fatal or life-threatening adverse event that is unexpected and assessed by the Investigator to be possibly related to the use of Pulmozyme. An unexpected adverse event is one that is not already described in the Pulmozyme investigator Brochure. Such reports are to be telephoned or faxed to the FDA, Genentech, and IRB within 7 calendar days of first learning of the event. A copy of the report will be submitted to the NEI Program Official within 24 hours of FDA notification.

8.7.1.2 15 Calendar Day Written Report

The Investigator will notify the FDA, and IRB in a written IND Safety Report, of any serious, unexpected AE that is considered reasonably or possibly related to the use of Pulmozyme. An unexpected adverse event is one that is not already described in the Pulmozyme investigator brochure. A copy of the report will be submitted to the NEI Program Official.

Written IND Safety reports will include an Analysis of Similar Events in accordance with regulation 21 CFR § 312.32. All safety reports previously filed by the investigator with the IND concerning similar events will be analyzed and the significance of the new report in light of the previous, similar reports commented on.

Written IND safety reports with Analysis of Similar Events will be submitted to the FDA and Genentech within 15 calendar days of first learning of the event. The FDA prefers these reports on a MedWatch 3500 form, but alternative formats are acceptable (e.g., summary letter).

8.8 Fax number for IND Safety Reports:

All written IND Safety Reports submitted to the FDA by the Investigator will also be faxed to Genentech Drug Safety, and to the IRB at UIC.

FDA: 1 (800) FDA 0178

Genentech Drug Safety: (650) 225-4682 or (650) 225-4630

IRB (UIC): (312) 413-2929

8.9 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 blind will be broken for ADR reports that are Serious and Unexpected The CRF and the source document for the subject must describe the departure from the protocol and state the reason.

8.10 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.

8.11 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, especially those with ocular GVHD. 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.

8.12 Reporting Changes in Study Status

During the funding of this study, any action by the FDA, an IRB, the industry collaborator (Genentech), or one of the study investigators that results in a temporary or permanent suspension of the study will be reported to the NEI Program Official within 3 business days of notification.

9 Data Quality and Safety Review Plan and Monitoring

9.1. *Data Quality and Management*

9.1.1. ***Plan for Data Quality and Management:*** The PI or study staff will review all data collection forms on an ongoing basis for data completeness and accuracy as well as protocol compliance.

9.1.2. ***Frequency of Data Review for this Study:*** The frequency of data review for this study differs according to the type of data and can be summarized in the following Sample Table

Data type	**Frequency of review**	**Reviewer**
Subject accrual (including compliance with protocol enrollment criteria)	Semiannually	PI, Independent Monitor
Status of all enrolled subjects, as of date of reporting	Semiannually	PI, Independent Monitor
Adherence data regarding study visits and intervention	Semiannually	PI, Independent Monitor
AEs and rates (including out-of-range lab values)	Semiannually	PI, Independent Monitor
SAEs	Per occurrence	PI, Independent Monitor FDA, NEI Program Official

9.2. ***Subject Accrual and Compliance***

9.2.1. ***Measurement and Reporting of Subject Accrual, Compliance With Inclusion/Exclusion Criteria:*** Review of the rate of subject accrual and compliance with inclusion/exclusion criteria will occur semiannually to ensure that a sufficient number of participants are being enrolled and that they meet eligibility criteria and the targeted ethnic diversity goals outlined in the grant proposal.

9.2.2. ***Measurement and Reporting of Participant Adherence to Treatment Protocol:*** Data on adherence to the treatment protocol will be collected by research staff and reviewed semiannually by the PI and the Independent Medical Monitor. Adherence of participants will be evaluated by performing eyedropper counts and by reviewing patient diaries at each visit. If adherence falls below 75%, which might inhibit the ability of the study to test its primary hypotheses, the Independent Medical Monitor will suggest a conference call for study investigators to discuss methods for improving adherence.

9.3. ***Stopping Rules:*** This study will be stopped prior to its completion if: (1) the intervention is associated with adverse effects that call into question the safety of the intervention; (2) difficulty in study recruitment or retention will significantly impact the ability to evaluate the study endpoints; (3) any new information becomes available during the trial that necessitates stopping the trial; or (4) other situations occur that might warrant stopping the trial. The PI will include an assessment of futility in the annual progress report to NEI and will consult with the study monitors to assess the impact of significant data loss due to problems in recruitment, retention, or data collection.

9.4 ***Data and Safety Monitoring Board (DSMB):*** The PI has designated a Data and Safety Monitoring Board to perform an independent review of ongoing study progress and safety. The Monitoring Board for this study is comprised of three voting members: Dr. Timothy McMahon who is also the independent monitor, Joelle Hallak. and Larry Tobacman. Dr. McMahon, Hallak, and Tobacman are not associated with this research project and thus work independently of the PI, Dr. Sandeep Jain. They are not part of the key personnel involved in this grant. They are qualified to review the patient safety data generated by this study because of their unique expertise in the area of clinical trials, ophthalmological diseases and epidemiology/biostatistics. Dr. Hallak, who is the Director of the UIC Center for Ophthalmology Clinical Trials and Translational Studies (CO-CTTS), has experience in epidemiologic studies, therefore will be the chairperson of the DSMB. Dr. McMahon, Hallak, and Tobacman view their role as representing the interests of the patients enrolled in the trial and not those of UIC. Drs. McMahon, Hallak, and Tobacman report no conflict of interest that includes financial interest or professional interest (in the sense of the trial outcome benefiting the individual professionally) or proprietary interest. The PI will be a non-voting ex-officio member of the DSMB. DSMB will meet at
least annually. Interim meetings may be held at the request of DSMB members, the study leadership, or the NEI.

9.4.1 *Responsibilities of the DSMB*

- Review and approve the research protocol(s) and plans for data and safety monitoring.
- Review interim analyses of outcome data and cumulative toxicity data for safety and efficacy to determine whether the trial should continue as originally designed, should be changed, or should be terminated.
- Review and approve the primary trial manuscript(s) with regard to determining that the results are fairly presented and the conclusions appropriate.
- Review proposed modifications to the study prior to their implementation
- Following each DSMB meeting, provide the study leadership and the NEI with written recommendations related to continuing, changing, or terminating the trial.

9.5 ***Safety Review Plan:*** Study progress and safety will be reviewed semiannually (and more frequently if needed). Progress reports, including patient recruitment, retention/attrition, and AEs will be provided to the Independent Medical Monitor following each of the semiannual reviews. An Annual Report will be compiled and will include a list and summary of AEs. In addition, the Annual Report will address (1) whether AE rates are consistent with pre-study assumptions; (2) reason for dropouts from the study; (3) whether all participants met entry criteria; (4) whether continuation of the study is justified on the basis that additional data are needed to accomplish the stated aims of the study; and (5) conditions whereby the study might be terminated prematurely. The Annual Report will be sent to the Independent Medical Monitor and will be forwarded to the DMSB, IRB, NEI Official, FDA, and industry collaborator. The PI will also send copies of recommendations and comments from the Independent Medical Monitor or Chair of the DSMB to the NEI Program Officer within 2 months of each monitoring review.

10 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.

11 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.

11.1 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.

12 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.

13 Study Finances

13.1 Funding Source

Departmental funds have been committed to the Corneal Neurobiology Laboratory. National Eye Institute, NIH is funding this research. We would expect National Eye Institute, NIH to have access to the information on site (here at UIC), without us transferring it.
Genentech is providing study drug and vehicle for the study for no cost but is not giving any funding. That is, Genentech is not providing any dollar support. 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).

13.2 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.

The University of Illinois owns a patent on some of the technology used in the drug being studied. If research shows the drug is safe and effective, the University of Illinois would receive a part of the profits from any sales of the drug. The Institutional Review Board believes that the possible financial benefit to the University is not likely to affect participant's safety in the study.

13.3 Subject Stipends or Payments

$50/ 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. The total amount of compensation will be $300.

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APPENDIX I

Genentech
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SAFETY REPORTING FAX COVER SHEET

Genentech Supported Research

AE / SAE FAX No: (650) 225-4682

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Reporter name	
Reporter Telephone #	
Reporter Fax #	

Initial Report Date	[DD] / [MON] / [YY]
Follow-up Report Date	[DD] / [MON] / [YY]

Subject Initials (Enter a dash if patient has no middle name)	[ ] - [ ] - [ ]

SAE or Safety Reporting questions, contact Genentech Drug Safety: (888) 835-2555

PLEASE PLACE MEDWATCH REPORT or SAFETY REPORT BEHIND THIS COVER SHEET