

A Phase I/II Randomized Placebo-Controlled, Double-Blind, Single-Center, Tolerability and Preliminary Efficacy Clinical Trial of Intravenous Immunoglobulin (IVIg) Eye Drops In Patients with Dry Eye Disease

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SCHEDULE OF VISITS AND PROCEDURES

	Screening	Day 1 (Pre-Dose)	Day 1 (Post-Dose)	Week 4	Week 8	Week 10
Informed consent	X					
Demographic information	X					
Medical history/ Con Meds	X					
Ophthalmic/ Dry Eye history	X					
Vital signs		X		X	X	X
Visual Acuity (Snellen's chart) with eye glasses (if applicable)		X		X	X	X
Ocular Surface Disease Index (OSDI)	X	X		X	X	X
Clinical Global Impression (CGI)				X	X	X
Subject Global Assessment (SGA)				X	X	X
Test Substance Tolerance (VAS)			X	X	X	
Ophthalmic examination (Slit lamp)	X	X		X	X	X
Schirmer I test	X	X			X	
Lissamine Dye staining	X	X			X	
Ocular surface Redness (OR) score	X	X		X	X	X
Ocular surface wash collection for analysis		X			X	
Keratograph Oculus Redness Score		X			X	
Non-invasive Keratography Tear Film Break-up Time (NIK BUT)		X			X	
Symptom Intensity Assesment		X		X	X	X
InflammaDry for MMP-9 Protein		X			X	
Pregnancy test	X					
Randomization visit		X				
First study medication		X				
Drug dispensation visits			X	X		

Instruction on study medication self-administration			X			
Adverse Events/ Concomitant Medications		X	X ⁺⁺	X	X	X

*If applicable ++ AEs only

ABBREVIATIONS

AE	Adverse Event
APC	Antigen-presenting cell
ACPA	Anti-Citrullinated Protein Antibodies
BAK	Benzalkonium Chloride
CNL	Corneal Neurobiology Laboratory
CRF	Case Report Form
CGI	Clinical Global Impression
CGA	Clinical Global Assessment
CONSORT	Consolidated Standards of Reporting Trials
DED	Dry Eye Disease
DREAM	Dry Eye Evaluation and Management
DTS	Dysfunctional Tear Syndrome
EC	Ethics Committee
EEL	Eye and Ear Infirmary
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act
ICH	International Conference on Harmonization
IB	Investigator's Brochure
IOP	Intraocular pressure
IRB	Institutional Review Board
IgA	Immunoglobulin A
IgG	Immunoglobulin G
KCS	Keratoconjunctivitis sicca
LFU	Lacrimal Functional Unit
NET	Neutrophil extracellular trap
NIKBUT	Non-invasive Keratograph Tear Film Break-Up Time
oGVHD	ocular Graft-vs-Host Disease
OSD	Ocular Surface Disease
OSDI	Ocular Surface Disease Index
OTC	Over the counter
BID	Two times per day
IVIG	Intravenous Immunoglobuline
SAE	Serious Adverse Event
SANDE	Symptom Assessment in Dry Eye
SGA	Subject Global Assessment
SPEED	Standardized Patient Evaluation of Eye Dryness
SS	Sjogren Syndrome
UIC	University of Illinois at Chicago
VA	Visual Acuity
VAS	Visual Analog Scale
VBR	Validated Bulbar Redness

Study Summary

Title	A Phase I/II Randomized Placebo-Controlled, Double-Blind Single-Center, tolerability and preliminary efficacy clinical trial of Intravenous Immunoglobulin (IVIG) eye drops in patients with Dry Eye Disease
Short Title	IVIG-eye drops treatment for Dry Eye Disease
Protocol Number	
Phase	Phase I/II
Methodology	Double-blind
Study Duration	10 weeks
Study Center(s)	Single-center
Objectives	To evaluate the safety and preliminary efficacy of IVIG-eye drops in patients with Dry Eye Disease.
Number of Subjects	24 (study drug- 12, placebo- 12)
Diagnosis and Main Inclusion Criteria	Men and women ≥ 18 years of age with a diagnosis of Dry Eye Disease, with Schirmer's test ≥ 0 to ≤ 9 mm/5min, Ocular surface staining ≥ 1 and OSDI mild (≥ 13), with high ACPA (> 4.4 units) in ocular surface wash
Study Product, Dose, Route, Regimen	<u>Study drug</u> : Intravenous Immunoglobulin (IVIG), 4 mg/ml (0.4%) eye drops two times a day for eight weeks. <u>Control</u> : Normal Saline Eye Drops (0.9% NaCl)
Duration of administration	8 weeks
Reference therapy	Normal Saline eye drops two times a day for eight weeks.

Statistical Methodology	<p>Based on the classification of reasons for conducting pilot studies by Thabane et al, in this pilot study we will assess (i) Process– recruitment rate, retention rate, (non)compliance or adherence rate; and (ii) Scientific– safety (adverse event/SAE rate), tolerability, and efficacy, including reduction in clinical signs and symptoms (OSDI, Corneal staining, Redness etc) and treatment effect size. We will calculate the observed rate with 95% confidence interval for each rate by treatment group. Other descriptive statistics, including median with interquartile or mean with standard deviation for continuous variables and frequency with percentage for categorical variables will be reported as well. To estimate the preliminary efficacy under the scientific category, one eye (target eye) will be selected at screening visit 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 corneal stain score is used; (iii) if both eyes have the same corneal stain 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. Due to the nature of this feasibility study, any efficacy statistical tests and adjusted analyses will not be conducted. Detailed data validation will examine completeness, existence and accuracy of collected data to assess data quality and identify missing and conflicting data. Statistical analysis will be performed on an intention-to-treat basis i.e., inclusion of patients randomly assigned, regardless of adherence, actual treatment received, and subsequent withdrawal of treatment and/or deviation from the protocol and per-protocol (i.e., inclusion of those who completed the treatment as planned)¹ and will be reported according to 2010 CONSORT guidelines.² All statistical analyses will be completed using SPSS Statistics V.22.0.31</p>
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1 Background & Rationale

1.1 Introduction

The sponsor-investigator (Sandeep Jain, MD) is initiating clinical investigations using Intravenous Immune Globulin (IVIG) eye drops 0.4% for the treatment of Dry Eye Disease (DED) in patients who have demonstrated presence of anti-citrullinated protein antibodies (ACPAs > 4.4 units in ocular surface wash) over the ocular surface. IVIG-eye drop is a fractionated plasma product comprising Immunoglobulin G (IgG) that offers significant advantages over unlicensed blood-derived eye drops (unfractionated serum or plasma eye drops). IVIG is currently an FDA approved product that is available from Grifols USA, LLC as Flebogamma 5% DIF immune globulin intravenous (human), indicated for treatment of primary (inherited) immunodeficiency (PI) in adults and pediatric patients 2 years of age and older. Flebogamma 5% DIF will be repurposed as IVIG-eye drops by diluting in normal saline to achieve 4mg/ml (0.4%) dose.

We have discovered that citrullinated proteins are present on the ocular surface of patients with DED and further discovered that anti-citrullinated protein antibodies (ACPA) are also present in the tear fluid of these patients (**Figure 3**). We also discovered that receptors (Fc-gamma 1) for these IgG autoantibodies are expressed on the ocular surface of neutrophils and dendritic cells and found evidence of engagement of IgG with Fc-gamma 1 receptors (**Figures 6 & 7**). Based on these findings, we hypothesized that IgG autoantibodies in the tear fluid contribute to ocular surface inflammation via interaction with Fc-gamma 1 receptors. In a murine model, we demonstrated that topical application of ACPA causes ocular surface disease (**Figure 12 A1, A2**) and competition with topical IgG abrogates surface disease (**Figure 12 A3, A4**). We also found that a topical application of IVIG-eye drops (4mg/ml) to murine eye does not cause ocular surface toxicity (**Figure 9**). ***Given these findings, we propose a feasibility clinical trial using IVIG eye drops to treat ocular surface disease in patients with DED who have high ACPA in the tear fluid.***

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.

1.2 Dry Eye Disease: Definition and Pathogenesis

Dry eye is a multifactorial disease of the ocular surface characterized by a loss of homeostasis of the tear film, and accompanied by ocular symptoms, in which tear film instability and hyperosmolarity, ocular surface inflammation and damage, and neurosensory abnormalities play etiological roles.³ DED is also recognized as keratoconjunctivitis sicca (KCS), sicca syndrome, keratitis sicca, xerophthalmia, dry eye syndrome (DES), dysfunctional tear syndrome (DTS), ocular surface disease (OSD) or dry eye. DED is caused by chronic instability of preocular tear film. Tear film instability can be triggered by insufficient tear production, or by poor tear film quality that results in increased tear evaporation^{4, 5}. Furthermore, dry eye is typically categorized into two groups:

- 1) Aqueous tear deficient dry eye disease
- 2) Evaporative dry eye disease

As DED progresses, lacrimal gland obstruction, meibomian gland orifice obstruction, thickened eyelid margins, cloudy, solid, or granular meibum secretion, eyelid telangiectasia, and meibomian gland

dysfunction become common clinical features. In advanced cases, dry eye can cause fibrotic thickening of the cornea and conjunctiva, filamentous keratitis, mucoid clumping, trichiasis, symblepharon, keratinization of the eyelids and meibomian glands, corneal and conjunctival erosion and thinning, corneal and conjunctival neovascularization, corneal and conjunctival scarring, corneal ulceration, and corneal perforation. In addition, prolonged ocular surface inflammation can lead to moderate or absolute loss/atrophy of the meibomian glands, lacrimal glands, and conjunctival goblet cells, and subsequently a dramatic reduction in tear film production and the onset of permanent DED⁶.

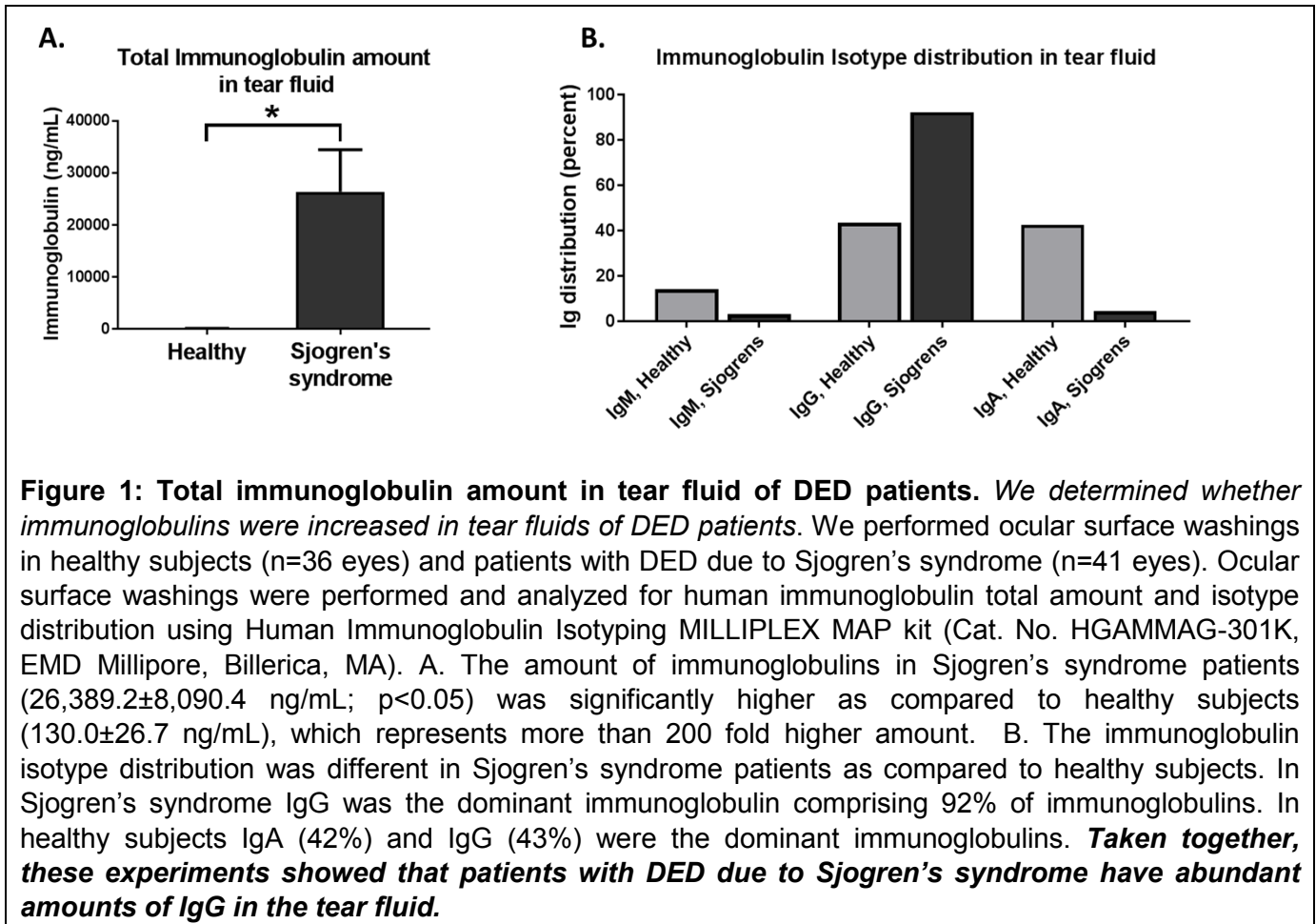
DED prevalence increases with age. The most common causes of dry eye are contact lens usage, autoimmune disorders, systemic drug effects, and refractive surgeries, particularly in middle-aged and older adults. DED also occurs in a higher percentage of women than men, especially in women entering menopause or pregnancy; hormone imbalances during menopause or pregnancy can cause lacrimal gland and ocular surface inflammation and tear film abnormalities⁴.

1.3 Dry Eye Disease: Pathogenesis – Autoimmune Inflammation

DED is a result of changes to the lacrimal functional unit (LFU). The LFU is composed of the lacrimal glands, cornea, eyelids, meibomian glands, conjunctiva, goblet cells, and ocular nerves. The LFU is responsible for the sustained production of adequate tear film to consistently lubricate the ocular surface. Structural changes to the LFU can induce tear film instability and insufficiency, which in turn can lead to tear hyperosmolarity. Chronic osmotic stress from tear film can activate stress associated pathways in ocular surface epithelial cells, thereby triggering a pro-inflammatory response that involves a mix of chemokines, cytokines, and matrix metalloproteinases. The subsequent maturation of antigen-presenting cells (APC's) on the ocular surface leads to the migration, activation, and expansion of autoreactive T cell lymphocytes as well as other leukocytic classes in the LFU. The constant recruitment of pro-inflammatory leukocytes onto the ocular surface inflicts epithelium damage in the form of small abrasions and epithelium barrier defects^{7, 8}. These abrasions can eventually progress to superficial punctate keratitis, squamous metaplasia, extracellular matrix deposits, decreased goblet cell differentiation, increased epithelial cell turnover (epitheliopathy), and significant ocular surface nerve damage and neuropathy.

1.3.1 Immunoglobulins are increased in tear fluid of Dry Eye Disease patients:

Immunoglobulins are a principal tear proteins that are directly secreted by lacrimal glands along with lactotransferrin, epidermal growth factor (EGF) and tear lipocalin.⁹ Some tear proteins are derived from serum, such as albumin, transferrin, IgG and IgM probably as a result of leakage from the blood vessels. It is also known that cell infiltrating conjunctiva (plasma cells) secrete immunoglobulins and cytokines in various conditions. There is general agreement that IgA is the pre-dominant tear immunoglobulin.¹⁰ IgA concentration in tears varies between 0.14 mg/ml – 0.40 mg/ml. IgG concentration in tears collected from the ocular surface is much less than IgA, reported as 0.02 mg/ml or undetectable,¹¹ although tears extracted from Schirmer strips have higher IgG amounts (0.23 mg/ml).¹² Therefore the IgA/IgG ratio in normal tears is >1.0. ***Our clinical data shows that patients with DED have abundant amounts of immunoglobulins in the tear fluid and the predominant immunoglobulin is IgG (Figure 1).***



1.3.2 Immunoglobulins are produced locally by ocular surface cells:

The human conjunctiva and lacrimal drainage system have an associated lymphoid tissue that is capable of detecting antigens and inducing a complete immune response by the activation of lymphatic cells and the production of antibodies.¹³ Antibodies in tears are not just the filtration product from the blood vessels, but are secreted by the immune tissue of the lacrimal system itself. In fact, in a quest for a needle-free vaccine administration strategy, ocular conjunctiva has been evaluated as an alternative mucosal immunization route. Conjunctival immunization with tetanus toxoid (TTd) induced TTd-specific local and systemic immune responses.¹⁴ Topical conjunctival application of ovalbumin also produces IgG antibodies in the tears.¹⁵ *Our clinical data shows the presence of T cells and B cells in close proximity amongst ocular surface cells (Figure 2A), suggesting active immune processing. Our data also shows the presence of IgG containing Plasma cells on the ocular surface (Figure 2B), suggesting antibody production and secretion by Plasma cells. Taken together, our data shows that all cellular players that are needed for inducing an immune response are present on the surface of the eye.*

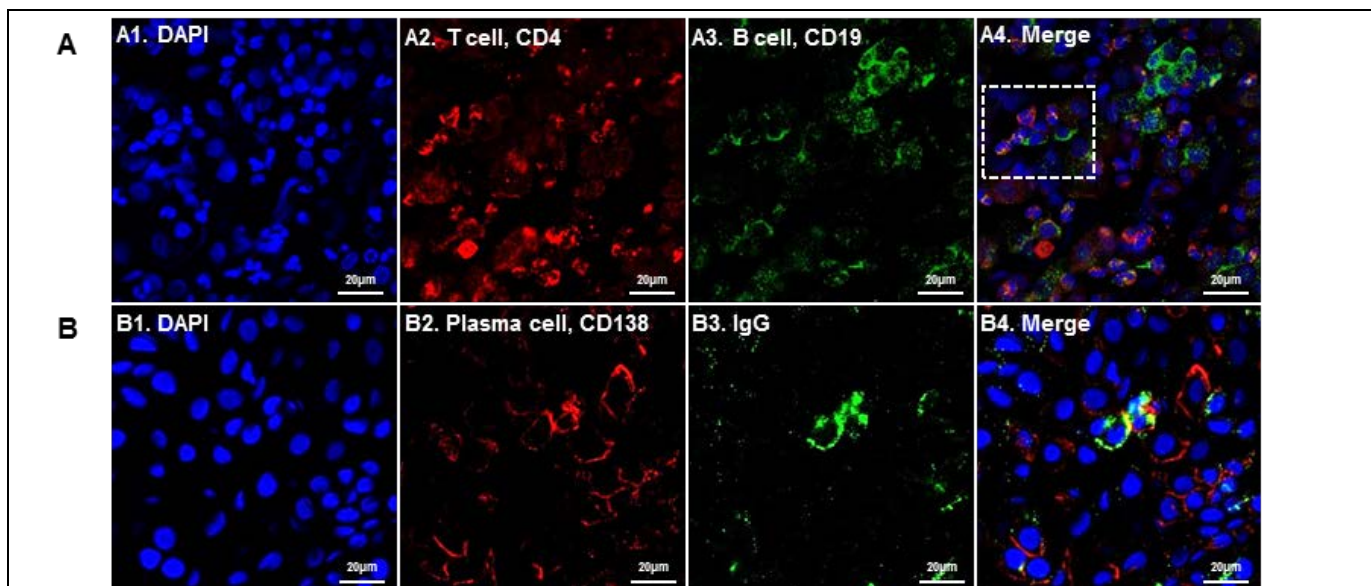


Figure 2: Immunofluorescence staining of Plasma cells and Immunoglobulins (IgG) in conjunctival impression cytology specimen obtained from patient with DED due to Sjogren's Syndrome. Conjunctival impression cytology was performed on Sjogren's Syndrome patients to determine whether the cellular components necessary for immune response are present on the ocular surface. These cells include antigen processing B cells (CD19 positive), T-helper cells (CD4 positive) and immunoglobulin secreting Plasma cells (CD138). Impression filter papers were processed for immunofluorescence staining with anti-CD4 antibody (mouse monoclonal anti CD4 antibody; R&D Systems, #MAB379, 1:50), anti-CD19 antibody (rabbit monoclonal anti CD19 antibody; Abcam, #ab134114, 1:20), anti-CD138 antibody (mouse monoclonal anti CD138 antibody; Bio-Rad, MCA2459GA, 1:500), and anti-IgG antibody (rabbit monoclonal anti human IgG antibody; Abcam, #ab109489, 1:500) and confocal immunofluorescence microscopy was performed to localize the proteins. Figure 2A2 shows the T-helper cells (red) and figure 2A3 shows the B cells (green). ***The T cells and B cells are in close proximity (inset box in 2A4), suggesting active immune processing.*** Figure 2B2 shows the presence of Plasma cells (red) and figure 2B3 shows the presence of IgG (green). ***Plasma cell and IgG co-localize (figure 2B4), suggesting that there may be antibody production and secretion by Plasma cells on the ocular surface of the patients with DED due to Sjogren's Syndrome.***

1.3.3 Autoantibodies are present in tear fluid in ocular surface disease:

Autoantibodies have been detected in tears of patients in the context of ocular diseases. Anti-HSV IgG is present in tear fluid of patients with HSV keratitis and the presence of anti-HSV IgG in tears is significantly associated with decreased corneal sensation, presence of stromal opacities, and with neurotrophic keratitis.¹⁶ In Chlamydia trachomatis, the leading global cause of preventable blindness, significantly higher anti-chlamydial protein IgG levels are found in the tear fluid of inflammatory trachoma cases as compared with levels in the controls.¹⁷ Several autoantibodies (anti-SS-A, anti-SS-B, and anti-DNase I) are present in tear fluid of Sjogren syndrome (SS) patients. These autoantibodies (anti-SS-A and anti-SS-B) are present in tears even though they are not detectable in the serum of Sjogren's syndrome patients, suggesting that anti-SS-A or anti-SS-B IgG synthesis may occur within lacrimal glands/ocular surface tissues.¹⁸ ***Our clinical data shows the presence of citrullinated proteins over the ocular surface and the presence of anti-citrullinated protein antibodies***

(ACPAs) in the tear fluid of several DED subtypes (Figure 3). We are the first to discover the presence of ACPAs in tear fluid of DED patients.

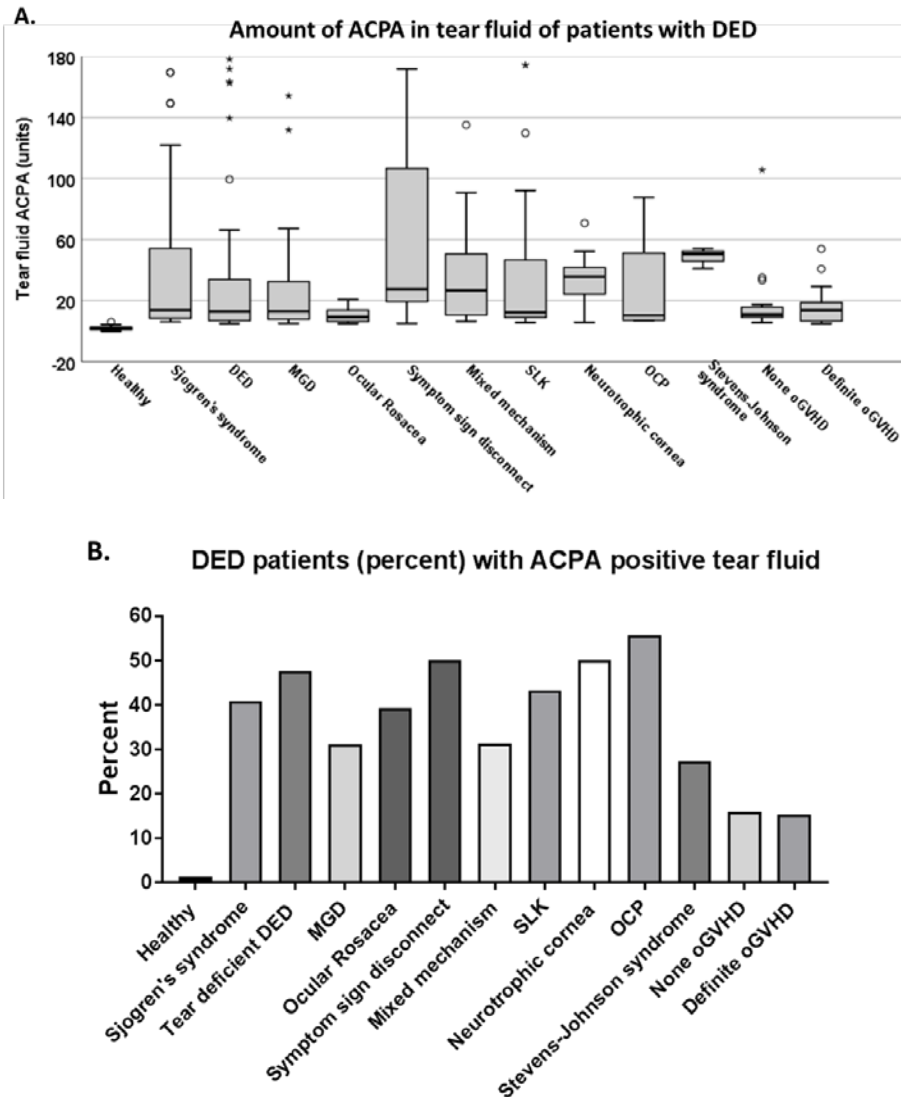


Figure 3: Presence of anti-citrullinated protein autoantibodies (ACPAs) in tear fluid of patients with DED. We determined the amount of ACPAs in the tear fluid of patients with DED and healthy subjects. Ocular surface washings were performed and analyzed for the presence and amount of ACPA using a semi-quantitative enzyme-linked immunosorbent assay (Quanta Lite CCP 3.1, Inova Diagnostics, San Diego, CA). Based on a 98th percentile value of ACPA amount in healthy subjects, we determined 4.4 units of ACPA to be the cutoff threshold above which values were considered positive for the presence of ACPA in tear fluid. We calculated the ACPA amount (units, median) in tear fluid of eyes that were positive for ACPA (i.e. ACPA > 4.4 units) and further calculated the percent of eyes that were ACPA positive (i.e. ACPA > 4.4 units) for each DED subtype. ACPAs were present in the tear fluid of many DED subtypes such as: (i) Sjogren's syndrome (13.8 units median in 40.9% of 54 eyes); (ii) tear deficient non-Sjogren's DED (13.0 units median in 47.8% of 54 eyes); (iii) meibomian gland dysfunction (MGD) (13.1 units median in 31.1% of 33 eyes); (iv) ocular rosacea (9.2 units median

in 39.3% of 11 eyes); (v) symptom sign disconnect (27.5 units median in 50.0% of 20 eyes); (vi) mixed mechanism (26.7 units median in 31.3% of 15 eyes); (vii) Superior limbic keratoconjunctivitis (SLK) (12.4 units median in 43.3% of 29 eyes); (viii) neurotrophic cornea (35.7 units median in 50% of 14 eyes); (ix) ocular cicatricial pemphigoid (OCP) (10.4 units median in 55.6% of 10 eyes); (x) Steven Johnson syndrome (50.6 units median in 27.3% of 3 eyes); (xi) none oGVHD (10.8 units median in 16% of 20 eyes) and (xii) definite oGVHD (13.7 units median in 15.3% of 21 eyes). **Taken together, these data show that several DED subtypes have presence of ACPA in the tear fluid.**

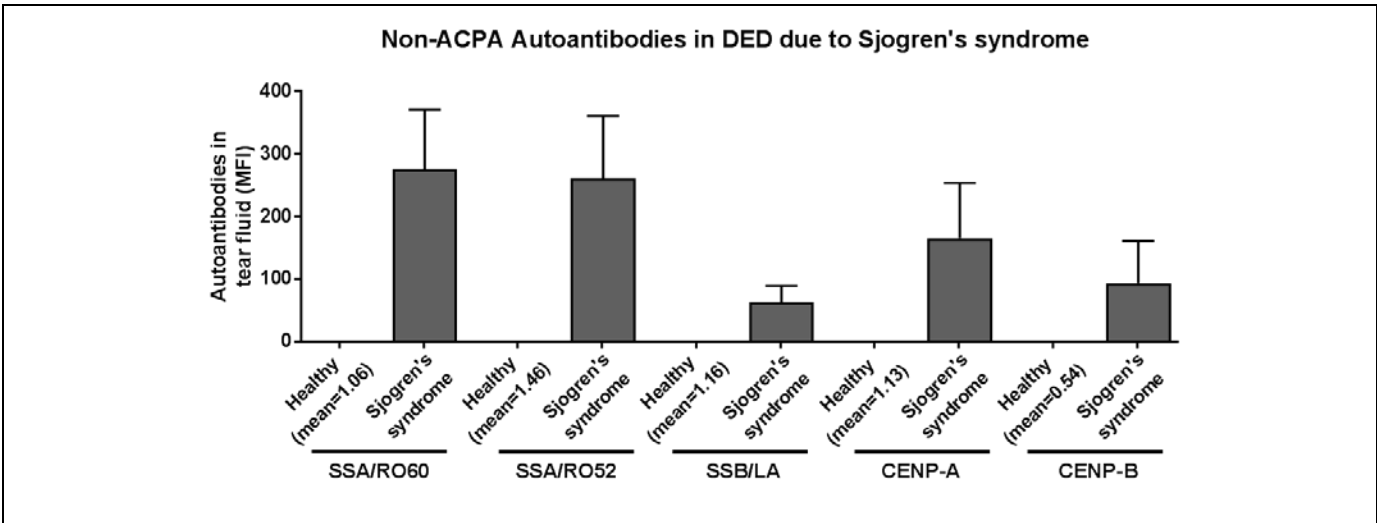


Figure 4: Presence of non-ACPA autoantibodies in DED due to Sjogren’s syndrome. We determined whether autoantibodies other than ACPAs were also present in tear fluid of patients with Sjogren’s syndrome. We performed ocular surface washings in healthy subjects (n=14 eyes) and patients with DED due to Sjogren’s syndrome (n=27 eyes). Ocular surface washings were analyzed for the presence of non-ACPA autoantibodies using Human Autoimmune Autoantibody MILLIPLEX MAP kit (Cat. No. HAIAB-10K, EMD Millipore, Billerica, MA). Several autoantibodies were detected: (i) SSA/RO60 (260 folds higher amount than healthy); (ii) SSA/RO52 (178 folds higher amount than healthy); (iii) SSB/LA (54 folds higher amount than healthy); (iv) CENP-A (147 folds higher amount than healthy) and (v) CENP-B (173 folds higher amount than healthy). **This data shows that several autoantibodies were present in the tear fluid of Sjogren’s syndrome patients.**

1.4 Dry Eye Disease: Clinical Signs and Symptoms

Common signs and symptoms of DED include: eye redness, ocular pain, burning and stinging sensation, foreign body sensation, pruritus, itchy or scratchy eye sensation, tired eyes, enhanced eye pressure, photophobia, painful mucous discharge, and in some cases epiphora. DED typically affects eyes bilaterally. Dry eye can heavily impact visual function especially during visually intensive activities and can overall decrease quality of life.^{4, 6} **Our clinical data shows that patients who have ACPAs in the tear fluid have more severe signs of DED (Table 1).**

Group	ACPA in tear fluid (units)	CCP in serum (units)	Signs			
			Schirmer I (median)	Corneal staining (median)	Conjunctival staining (median)	Corneal melts/scars (% eyes)
ACPA+/CCP+	148.0	160.0	1.0	5.0	4.0	25
ACPA+/CCP-	14.0	4.0	7.0	2.0	2.0	12.4
ACPA-/CCP-	-1.0	4.0	8.0	1.0	2.0	9.4
p value	<0.001	<0.001	<0.001	0.020	0.027	--

Table 1: Comparison of clinical signs and symptoms of DED in patients who have presence of anti-citrullinated antibodies in tear fluid (ACPA+) or serum (CCP+) with those who do not have these antibodies in tear fluid (ACPA-) or serum (CCP-). *It is known that rheumatoid arthritis patients who have ACPA antibodies in serum have more severe joint disease, therefore we determined whether patients who have presence of ACPA in tear fluid similarly have more severe ocular surface disease.* The presence of ACPA in tear fluid of DED patients (n=322 eyes) was determined using a semi-quantitative enzyme-linked immunosorbent assay (Quanta Lite CCP 3.1, Inova Diagnostics, San Diego, CA). Patients who had tear fluid ACPA>4.4 units were considered positive (ACPA+). The presence of ACPA in serum was determined by processing performed in clinical laboratory. Patients who had serum ACPA>20 units were considered positive (CCP+). Based on whether patients were ACPA+, ACPA-, CCP+ and CCP- patients were divided into 3 groups: (i) ACPA+/CCP+ (n=28 eyes). These patients had ACPAs in tear fluid as well as serum; (ii) ACPA+/CCP- (n=145 eyes). These patients had ACPAs in tear fluid but not in serum; and (iii) ACPA-/CCP- (n=149 eyes). These patients did not have ACPA in tear fluid as well as in serum. Patients with ACPA+/CCP+ had the highest levels of ACPA in tear fluid as well as in serum compared to other groups. Eyes in ACPA+/CCP+ group had more severe signs and symptoms of DED as compared to other groups. These eyes had more severe tear deficiency (Schirmer I) and more severe ocular surface disease (corneal staining and conjunctival staining). These eyes also had more corneal complications (ulcers, melts or scars). **Taken together, these data show that patients who have ACPAs in the tear fluid have more severe signs of DED.**

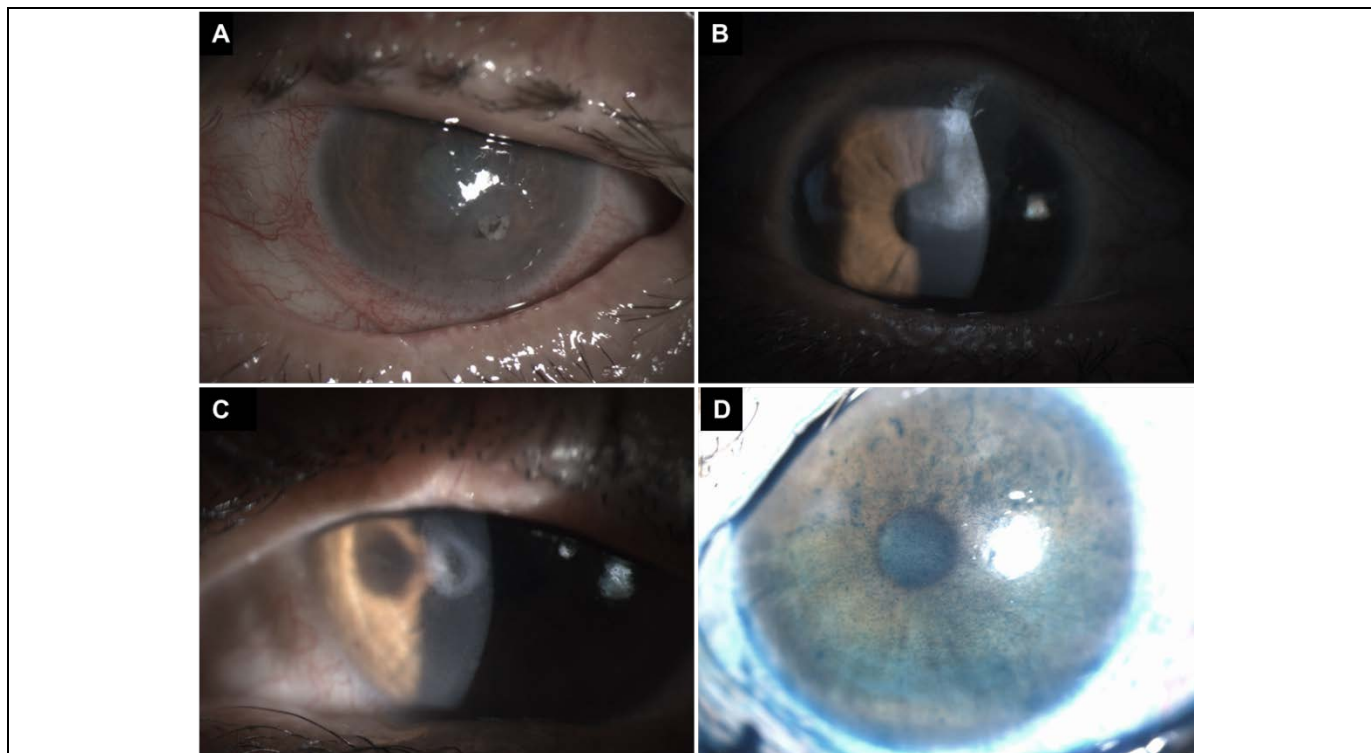


Figure 5. Photographs of eyes of Sjogrens syndrome patients with DED and high ACPA levels in tear fluid showing severe corneal disease. (A) 76 year old female with Sjogrens syndrome showing a corneal perforation. Serum ACPA level was high (137 units) and tear ACPA levels were also very high (145.9 units) in the eye with perforation. In the fellow eye, the ACPA levels were lower (55.8 units). **(B)** A 38 year old female with Sjogrens syndrome showing a corneal scar following sterile ulceration. Serum ACPA level was high (216 units) and tear ACPA levels in both eyes were also very high (121.8 units in the right eye and 142.6 units in the left eye). **(C)** A 50 year old female with Sjogrens syndrome showing a corneal melt resulting in a corneal divot. Serum ACPA level was high (117 units) and tear ACPA levels in both eyes were also very high. **(D)** A 64 year old female with Sjogrens syndrome showing severe ocular surface disease as evidenced by extensive lissamine green punctate staining of corneal epitheliopathy. Serum ACPA level was high (160 units) and tear ACPA levels in both eyes were also very high (153.6 units in both eyes). ***Taken together, these four cases demonstrate that very high levels of ACPA in tear fluid is associated with severe ocular surface disease.***

1.5 Dry Eye Disease: Diagnostic Testing

Clinicians use several diagnostic tests to diagnose DED and to assess disease severity. These tests fall into two groups: signs (objective) and symptoms (subjective). For DED signs, there are several quantitative tests, including: Schirmer Test, Schirmer I Test, Schirmer II test, tear film breakup time (TBUT), non-invasive tear film breakup time (NITBUT) epithelial staining scores (rose bengal, lissamine green, fluorescein) via slit-lamp examination, tear function index (TFI), tear fluid protein immunoassays, fluorophotometry, meibography, meibometry, meiboscopy, meniscometry, lacrimal gland biopsy, impression cytology, hypolysozyme measurement, hyperosmolarity measurement, and lipid layer analysis, ocular redness scoring, automated blink analysis, and meniscus evaluation utilizing Lipiview II, Keratograph 5M, or OCT equipment.⁴

Symptom measurements utilizing physician and/or patient disease scoring include: extensive dry eye questionnaire (DEQ), visual analog scale (VAS), ocular surface disease index (OSDI), national eye institute visual function questionnaire (NEI-VFQ-25), symptom assessment in dry eye questionnaire (SANDE), and standardized patient evaluation of eye dryness questionnaire (SPEED).^{19, 20}

Many of the above tests have been used in recent DED studies. In this feasibility study, there will be multiple exploratory endpoints based on conventional dry eye assessments, subjective and objective (section 2.4). Based on the results of this study, we will decide which sign and/or symptom to be used as the primary (or co-primary) endpoint(s) in later stage studies.

1.6 Dry Eye Disease: Current Management

Typically, clinicians prescribe artificial tear eyedrops and topical corticosteroids for short-term relief of DED. Antibiotics (tetracyclines and macrolides), non-steroidal anti-inflammatory agents, autologous serum drops, omega fatty acids, mucin secretagogues, and anti-inflammatory agents are also used to combat DED symptoms. In addition, prosthetic scleral lenses (i.e. PROSE) that also serve as supplemental tear reservoirs are increasingly being prescribed to enhance ocular surface hydration in patients with chronic DED. Hot eyelid compresses are often utilized to treat meibomian gland dysfunction, a primary driver of evaporative dry eye disease. In advanced cases of DED, punctual plugs can be installed to block tear drainage. In severe cases of dry eye, tarsorrhaphy surgery, tear duct cauterization, or amniotic membrane transplant might be required to reduce tear evaporation.^{4, 21}

Currently there are only two pharmaceutical agents that are FDA approved for the treatment of dry eye: cyclosporine an ophthalmic emulsion (Restasis®) and lifitegrast ophthalmic solution (Xiidra™). Restasis® 0.05% is a topical immunomodulator indicated to increase tear production in patients whose tear production is presumed to be suppressed due to ocular inflammation associated with keratoconjunctivitis sicca (Restasis® Prescribing Information). Xiidra™ 5% is a lymphocyte function-associated antigen (LFA-1) antagonist indicated for the treatment of signs and symptoms of dry eye disease (Xiidra™ Prescribing Information). Given the complexity, severity, and frequency of DED, and given the limited modes of action by which these two compounds treat dry eyes, there is a medical need for other dry eye therapies, particularly those with multiple modes of action that target the wider dry eye population and are effective and safe for long-term daily use.

1.7 Scientific Rationale for IVIG eye drops in Dry Eye Disease

There is considerable scientific rationale to establish a medically plausible basis for the use of IVIG eye drops for the treatment of DED. It is well established that autoimmune processes underpin ocular surface inflammation in DED. For example, B cell over-activation is a key feature of Sjogren's syndrome (a major cause of DED), attested by the wide spectrum of autoantibodies detected in these patients (e.g. antinuclear antibodies, anti-Ro/SSA, anti-La/SSB, rheumatoid factor).²² We, and others, have shown that autoantibodies are present in the tears of DED patients (**Figures 3 & 4**) that may contribute to ocular surface disease and inflammation (**Table 1**). IVIG has an immunomodulatory activity that is based on the modulation of biological processes that are implicated in innate or acquired immunity, therefore it has the potential to reduce autoimmune-mediated inflammation in DED.

To date, IVIG has not been applied to the ocular surface as eye drops. However, IVIG has been administered by the intravenous route to treat ocular surface diseases like ocular cicatricial pemphigoid (OCP) and several intraocular inflammatory diseases like birdshot retinochoroidopathy.²³⁻²⁵ Our data shows that the levels of IgG in the tear fluid increase approximately 3 folds after intravenous administration of IVIG to treat OCP (**Figure 13**). *We posit that topical application of IVIG-eye drops will achieve significantly higher levels of IgG over the ocular surface as compared to intravenous*

administration without any of the side effects associated with systemic administration of IVIG, without need for hospitalization for administration of the drug and at a fraction of the cost of IVIG.

We have shown in conjunctival impression cytology data that the receptors for autoantibodies (Fc gamma 1 receptor) are present on the cells (dendritic cells and neutrophils) in the superficial layers of the ocular surface, therefore these receptors are accessible to IVIG eye drops for exerting a therapeutic action (**Figures 6 & 7**). Pooled normal polyspecific human IgG (IVIG) contain antiidiotypic antibodies against human autoantibodies may provide a mechanism for the suppressive effect of IVIG in human autoimmune diseases. Whereas pooled IgG from a high number of normal individuals express antiidiotypic antibodies directed against autoantibodies, antiidiotypic antibodies are rarely found in IgG prepared from individual normal donors.^{26, 27} This implies IVIG eye drops will have anti-idiotypic antibodies but serum tear eye drops will not have antiidiotypic antibodies.

IVIG preparation contain pooled polyspecific IgG. Therefore, IVIG eye drops will increase the amount of IgG on the ocular surface. This is no different that applying serum tear eye drops to the ocular surface as serum contains IgG (approximately 10 mg/ml). In fact, applying IVIG eye drops 0.4% places 4 mg/ml IgG on the ocular surface, which is the identical amount of IgG placed on the ocular surface when using 50% serum tear eye drops. 50% serum tear eye drops have been used to treat a variety of ocular surface diseases and their use is widely accepted as a useful therapeutic strategy for severe ocular surface disease. Although autologous serum tears are more commonly used, there are several reports of use of allogeneic serum tears as well. We posit that use of IVIG 4 mg/ml will pose no more safety risk than that with use of 50% allogeneic serum tears that are already in clinical use.

The immunomodulatory biological effects of IVIG that may reduce the deleterious effects of autoantibodies in tear fluid include:

A. Functional Blockade of Fc Receptors. We have performed impression cytology of ocular surface in DED patients and found Fc receptor expressing neutrophils and mononuclear cells amongst ocular surface cells (**Figure 6**). We also found that IgG interacts with Fc gamma receptors that are expressed on neutrophils and dendritic cells on the ocular surface in DED patients (**Figure 7**). These IgG are likely to be autoantibodies. Blockade of Fc receptors by IgG in IVIG-eye drops may reduce the interaction of autoantibodies with Fc receptors, thus reducing the deleterious effects of autoantibodies on the ocular surface.

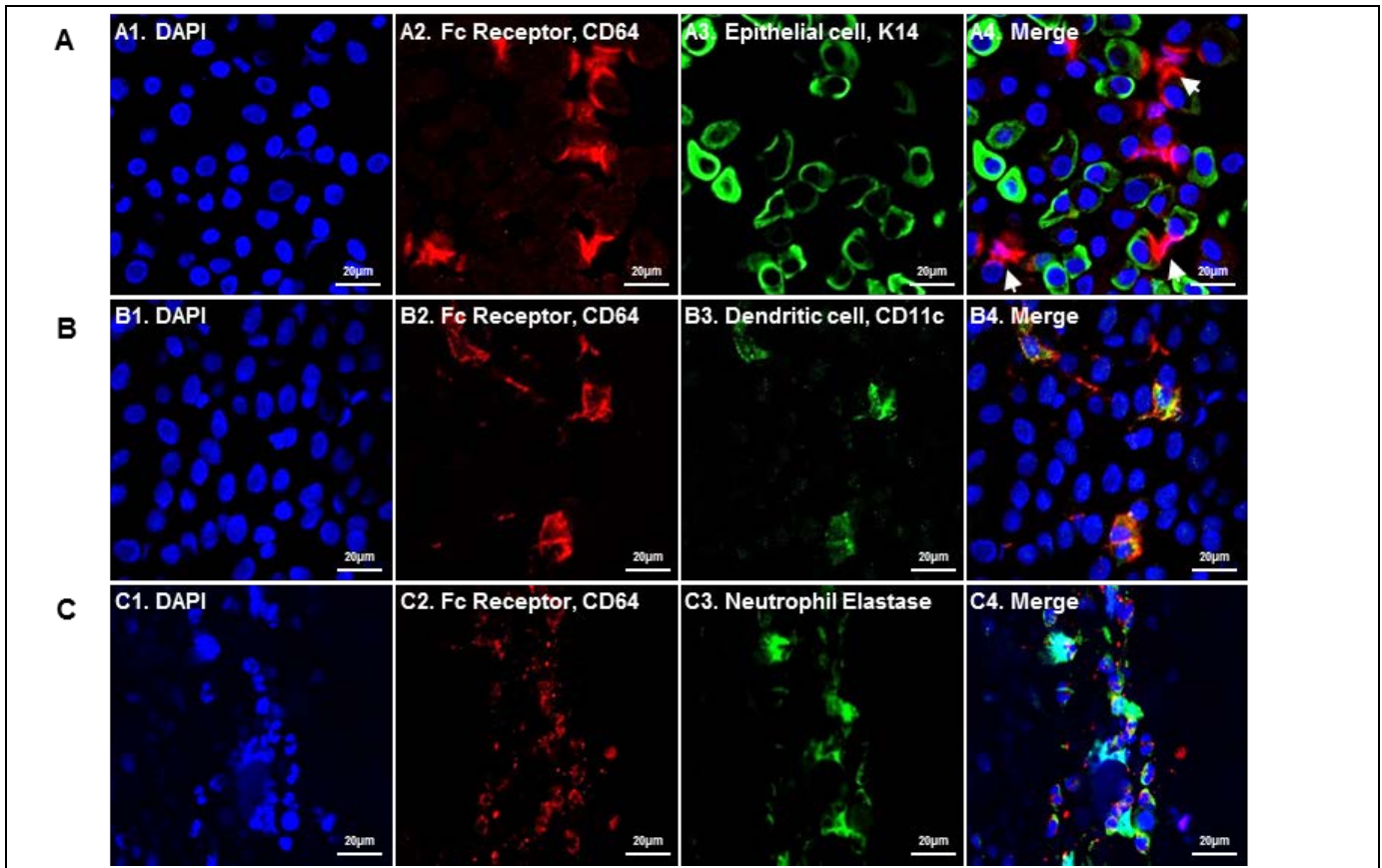


Figure 6: Immunofluorescence staining of Fc gamma 1 receptors and ocular surface cells in conjunctival impression cytology specimen obtained from patient with DED due to Sjogren's Syndrome. Since immunoglobulins (monomeric IgG or immune complexed IgG) bind to Fc gamma 1 receptors to propagate the immune response, the presence of Fc gamma receptors on ocular surface cells is a prerequisite for induction of an immune response. Therefore, we performed conjunctival impression cytology on Sjogren's Syndrome patients to determine whether Fc gamma 1 receptors (CD64 positive) are present on conjunctival epithelial cells (K14 positive) and bone marrow derived dendritic cells (CD11c positive) or neutrophils (neutrophils elastase positive) that are also present on the ocular surface. Impression filter papers were processed for immunofluorescence staining with anti-CD64 antibody (mouse monoclonal anti-CD64 antibody; Santa Cruz, #sc-1184, 1:500), anti-K14 antibody (rabbit polyclonal anti-cytokeratin 14 antibody; BioLegend, #905301, 1:1000), anti-CD11c antibody (rabbit monoclonal anti CD11c antibody; Abcam, #ab52632, 1:500), and anti-neutrophil elastase antibody (rabbit polyclonal anti neutrophil elastase antibody; Abcam, #ab21595, 1:500). Figure 6A2 shows expression of Fc gamma receptors (red) and figure 6A3 shows conjunctival epithelial cells (green). Figure 6A4 shows that Fc receptors and conjunctival epithelial cells do not co-localize. Therefore, Fc gamma receptors are expressed on non-epithelial cells of the ocular surface. Figure 6B2 shows expression of Fc gamma receptors (red) and figure 6B3 shows dendritic cells (green). Figure 6B4 shows co-localization of Fc gamma receptors on dendritic cells. Therefore, Fc gamma receptors are expressed on bone-marrow derived dendritic cells. Figure 6C2 shows expression of Fc gamma receptors (red) and figure 6C3 shows neutrophils (green). Figure 6C4 shows co-localization of Fc gamma receptors on neutrophils. Since neutrophils express Fc gamma receptors only when activated, expression of these receptors on neutrophils on the ocular surface suggests that these neutrophils are in an activated state. Taken together, figure 6 data shows that ***Fc gamma receptors (effectors for***

immunoglobulin induced immune reaction) are not expressed on conjunctival epithelial cells but are expressed on bone-marrow derived dendritic cells and neutrophils present on the ocular surface.

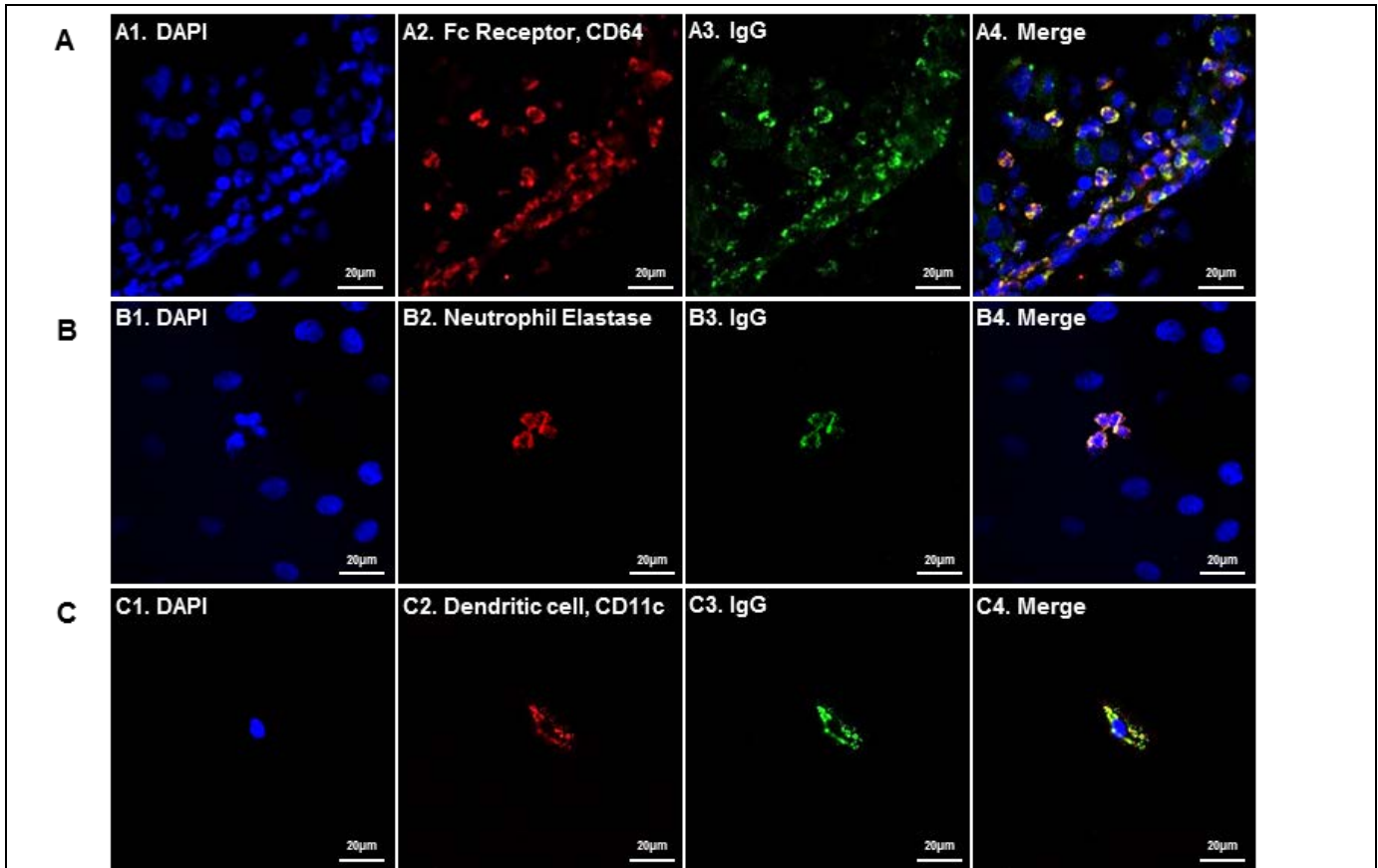


Figure 7: Immunofluorescence staining to demonstrate the interaction between immunoglobulins (IgG) and Fc gamma 1 receptors on ocular surface cells in conjunctival impression cytology specimen obtained from patient with DED due to Sjogren's Syndrome.

Since immunoglobulins bind to Fc gamma 1 receptors to propagate the immune response, the binding of IgG to Fc gamma receptors on ocular surface cells is a prerequisite for induction of an immune response. Therefore, we performed conjunctival impression cytology on Sjogren's Syndrome patients to determine whether IgG and Fc gamma 1 receptors (CD64 positive) co-localize and to determine which ocular surface cells bind to IgG. Impression filter papers were processed for immunofluorescence staining with anti-CD64 antibody (mouse monoclonal anti-CD64 antibody; Santa Cruz, #sc-1184, 1:500), anti-neutrophil elastase antibody (mouse monoclonal anti-neutrophil elastase antibody; Santa Cruz, #sc-53388, 1:500), anti-CD11c antibody (mouse monoclonal anti CD11c antibody; BD biosciences, #565805, 1:100) and anti-IgG antibody (rabbit monoclonal anti human IgG antibody; Abcam, #ab109489, 1:500) and confocal immunofluorescence microscopy was performed to localize the proteins. Figure 7A2 shows expression of Fc gamma receptors (red) and figure 7A3 shows IgG presence (green). Figure 7A4 shows that IgG co-localizes with Fc gamma 1 receptors, suggesting IgG-Fc gamma receptor interaction. Figure 7B2 shows presence of neutrophils (neutrophil elastase positive, red) and figure 7B4 shows these neutrophils co-localize with IgG. Figure 7C2 shows presence of bone-marrow derived dendritic cells (CD11c positive, red) and figure 7C4 shows these dendritic cells co-

localize with IgG. Taken together, figure 7 data shows that ***IgG interacts with Fc gamma receptors that are expressed on neutrophils and dendritic cells that are present on the ocular surface in DED patients in Sjogren's Syndrome.***

B. Autoantibody Neutralization and Inhibition of Autoantibody Production. IVIG preparations contain anti-idiotypic antibodies, i.e., antibodies that are able to interact specifically with the variable region (antigen recognition site) of autoantibodies. This interaction has the potential to neutralize an autoantibody and to hamper its production via binding to auto reactive B lymphocytes. Such a neutralizing or inhibitory activity has been shown, amongst others, for autoantibodies directed against factor VIII, DNA, intrinsic factor, thyroglobulin and anti-neutrophil cytoplasmic antibodies (ANCA). *We have found the presence of anti-citrullinated protein antibodies (ACPAs) in the tear fluid of DED patients (Figure 3). Anti-idiotypic antibodies in IVIG-eye drops may neutralize ACPAs in the tear fluid of DED patients to reduce ACPA-induced ocular surface disease.*

C. Other Mechanisms: (i). Complement Inhibition. The Fc portion of IVIG can bind the C3b and C4b fragments of complement, and thereby inhibit their tissue deposition as well as the generation of the C5 convertase (C4b2a3b), hampering the subsequent formation of the membrane attack complex. This cascade of events has been shown to occur in dermatomyositis and thereby contributes to the therapeutic effect of IVIG in that disease. (ii). Modulation of Cytokine and Cytokine Antagonist Production. IVIG may significantly modulate the production of several cytokines (including IL-1,-2,-3,-4,-5,-10, TNF-alpha, and GM-CSF) and cytokine antagonists (IL-1 receptor antagonist) by monocyte-macrophages and lymphocytes. (iii). Modulation of Dendritic Cell Properties. Recent work suggests that IVIG also affects the differentiation, maturation and functional status of dendritic cells (DCs). DCs appear to be a primary target for the immunosuppressive effects of IVIG on T-cell activation. (iv). Signaling through the Inhibitory Fc Receptor, Fcgamma RIIB. (v). Expansion of regulatory T cells and reciprocal inhibition of Th17 cells.

1.8 Specific Agent in this Study

The primary agent used in this study is a commercial Intravenous immunoglobulins (IVIG) formulation (Flebogamma DIF 5%) that will be diluted in 0.9% saline to achieve 4 mg/ml (0.4%) IgG concentration, and dispensed as eye drops. Intravenous immunoglobulins (IVIG) are a therapeutic preparation of pooled normal human IgGs obtained from large numbers of healthy donors.

Flebogamma DIF 5% IVIG is an FDA-approved product available as 50 mg/ml an immune globulin intravenous (human) solution indicated in adults and pediatric patients 2 years of age and older for the treatment of primary immunodeficiency (PI), including the humoral immune defects in common variable immunodeficiency, x-linked agammaglobulinemia, severe combined immunodeficiency, and Wiskott-Aldrich syndrome. It is stable at room temperature for more than two years.

Flebogamma 5% DIF contains 5 g human normal immunoglobulin and 5 g D-sorbitol (as stabilizer) in 100 mL of water for injection, and ≤ 3 mg/mL polyethylene glycol. There is no preservative in the formulation. The pH of the solution ranges from 5 to 6 and the osmolality from 240 to 370 mOsm/L, which is within the normal physiological range.

From a safety perspective, IVIG eye drops would most closely resemble allogeneic unfractionated blood eye drops (serum or plasma). From an efficacy perspective, IVIG eye drops have the potential to significantly improve outcomes of autoimmune and inflammatory ocular surface disease, given the long shelf life at room temperature making storage & dispensing by pharmacies possible.

IgG at 4 mg/ml (0.4%) concentration (in the form of serum/plasma 50% eye drops) is routinely used to treat ocular surface diseases, however widespread use is limited because of short shelf life and limited number of blood processing compounding pharmacies. We propose to use IgG at a similar 4 mg/ml (0.4%) concentration (in the form of IVIG eye drops) to treat ocular surface diseases as this strategy offers significant advantages, chief amongst which is a long shelf life at room temperature that makes widespread dispensing possible.

1.9 Safety Studies

We have performed *in vitro* studies using cultured human epithelial corneal cells to determine whether IVIG (4 mg/ml) causes cytotoxicity. Our data shows that IVIG 4 mg/mL does not induce cytotoxicity in cultured corneal epithelial cells (**Figure 8**).

We have performed *in vivo* studies to determine whether IVIG-eye drop (4 mg/mL) causes ocular surface toxicity when applied to murine corneas. Our data shows that there was no difference in corneal fluorescein staining between IVIG group and the control group. This suggests that topical application of IVIG eye drop (4 mg/mL) does not cause ocular surface toxicity (**Figure 9**).

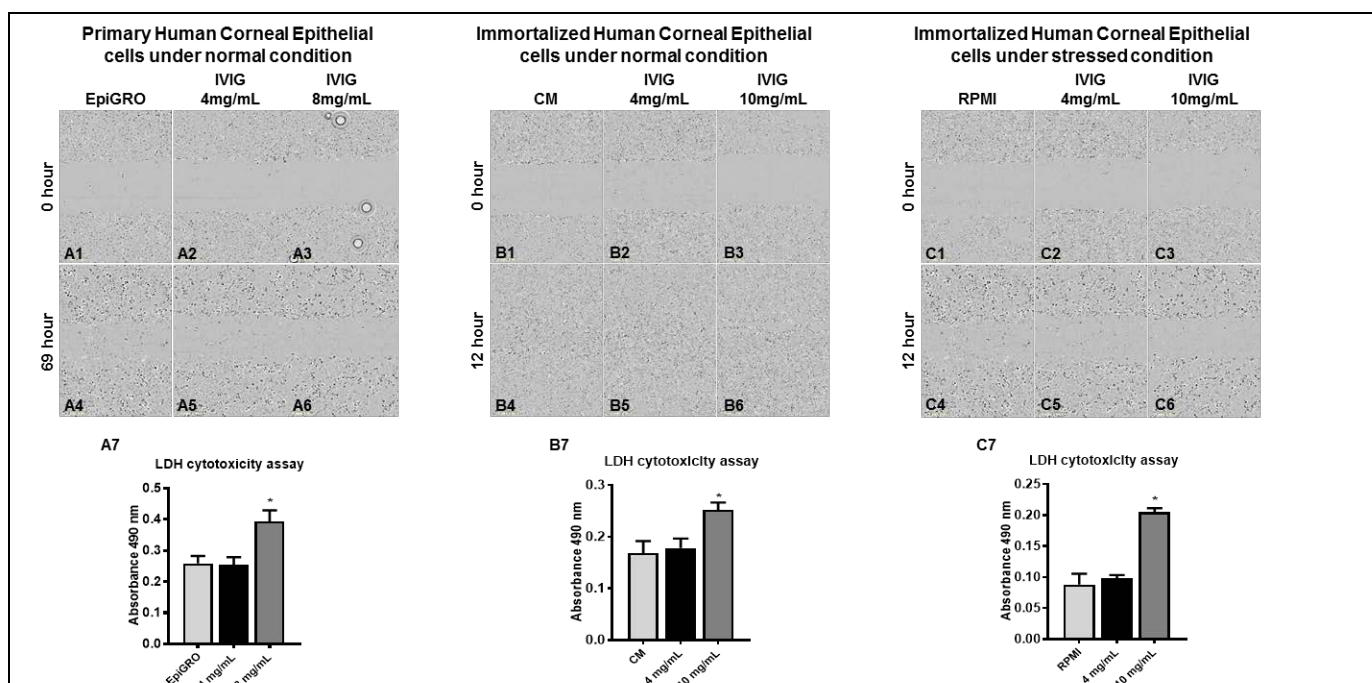


Figure 8: Wound scratch assay and LDH cytotoxicity assay in cultured human corneal epithelial cells (primary and immortalized). These experiments were performed to determine whether IVIG causes cytotoxicity in cultured human corneal epithelial cells. Human corneal epithelial cells were cultured under normal (serum) or stressed (serum free) conditions with or without incubation with IVIG (4 mg/mL or 10 mg/mL) in IncuCyte® S3 Live Cell Analysis System (Essen Biosciences). IVIG toxicity was assessed using the LDH assay kit from Thermo Scientific according to the manufacturer's specification. (A1-A6): Representative IncuCyte images after 69 hours of incubation show that scratch wounds in primary human corneal epithelial cells close similarly with or without IVIG. (A7) LDH cytotoxicity assay shows absence of cytotoxicity with 4 mg/mL IVIG but significant cytotoxicity with 8 mg/mL IVIG. Representative IncuCyte images after 12 hours of incubation show that scratch wounds in immortalized human corneal epithelial cells close similarly with or without IVIG under normal condition (B1-B6) as well as under stressed condition (C1-C6). LDH cytotoxicity assay shows absence of

cytotoxicity with 4 mg/mL IVIG but significant cytotoxicity with 10 mg/mL IVIG under both normal (B7) and stressed (C7) cultured conditions. **Taken together, these experiments show that IVIG 4 mg/mL does not induce cytotoxicity in cultured corneal epithelial cells.**

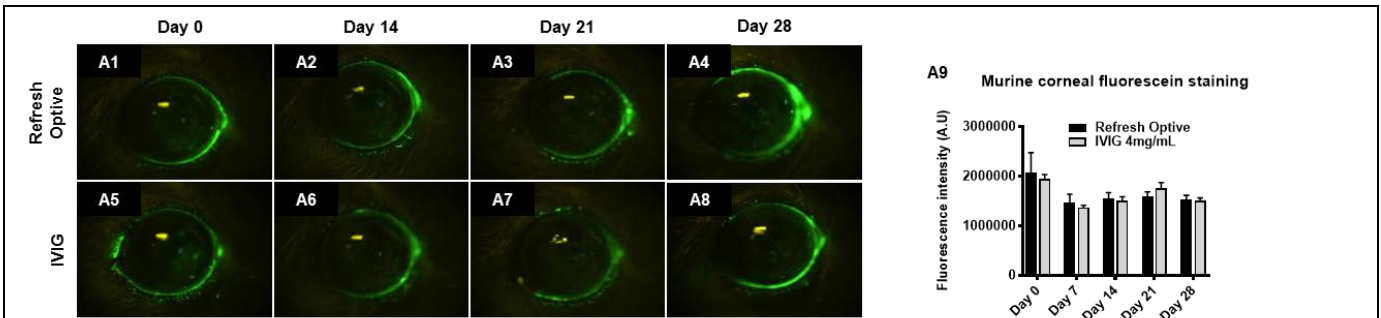


Figure 9: *In vivo* toxicology study of IVIG application in murine corneas. These experiments were performed to determine whether IVIG-eye drop (4 mg/mL) causes ocular surface toxicity when applied to murine corneas. IVIG (10 μ L of 4 mg/mL solution) or Refresh Optive (control) was applied once a day to the mouse corneas ($n=8$ /group) for 28 consecutive days. After each application, the mice were restrained for 1 minute to allow for adequate contact time of eye drop with the eye. Fluorescein staining was performed at base line and at weekly intervals thereafter. (A1-A8) Representative mouse cornea images showing fluorescein staining after incubation with Refresh Optive (control group, A1-A4) and IVIG (experimental group, A5-A8). (A9) Graph showing the quantitative data for the fluorescein staining at Day 0, 7, 14, 21 and 28. **There was no difference in fluorescein staining between IVIG group and the control group. This suggests that topical application of IVIG eye drop (4 mg/mL) does not cause ocular surface toxicity.**

1.10 Tissue Distribution Studies

We were unable to detect increased IgG levels in ocular tissues or serum after applying IVIG as eye drops in non-inflamed and inflamed murine eye. The poor penetration of IVIG eye drops in ocular tissues may actually be desirable because it further improve its safety profile for use in DED because DED is rarely associated with intraocular inflammation, therefore intraocular penetration of drug is not needed to treat DED.

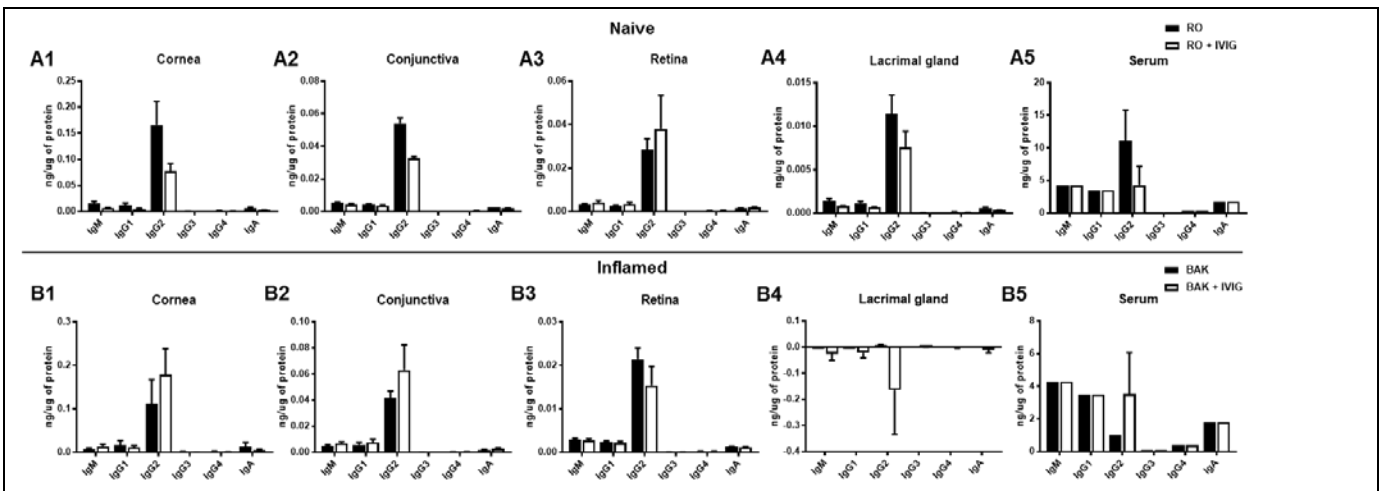


Figure 10: Tissue distribution study of IVIG application in naïve and inflamed murine corneas.

This experiment was performed to determine whether IVIG eye drops applied topically penetrate ocular tissues (cornea, conjunctiva, retina and lacrimal gland) in a naïve (A1-A5) or inflamed (B1-B5) eye.
Experiment 1 (Naïve eyes): Refresh Optive + IVIG (4 mg/mL) were applied to murine corneas (n=6/group) once a day for six consecutive days. Control eyes received Refresh Optive only.
Experiment 2 (Inflamed eyes): Benzalkonium chloride (BAK, 0.1%) + IVIG (4 mg/mL) were applied to the murine corneas (n=6/group) once a day for six consecutive days. Control eyes received BAK (0.1%) only. In both experiments, after each application, the mice were restrained for 1 minute to allow for adequate contact time of eye drop with the eye. After 6 days, the samples (cornea, conjunctiva, retina, extraorbital lacrimal gland and serum) were collected, normalized to protein amount, and processed for Luminex detection of Human IgG (name of the panel). (A1-A5): Graphs show human immunoglobulin isotypes collected from tissues in experiment 1 (naïve eyes). (B1-B5): Graphs show human immunoglobulin isotypes collected from tissues in experiment 2 (inflamed eyes). No tissue or serum showed a significant increase or decrease in human IgG amount. ***Taken together, these experiments show that topical application of IVIG does not reach intraocular tissues or serum.***

1.11 IVIG Animal Efficacy and Clinical Data to Date

1.11.1 IVIG Animal Efficacy Data

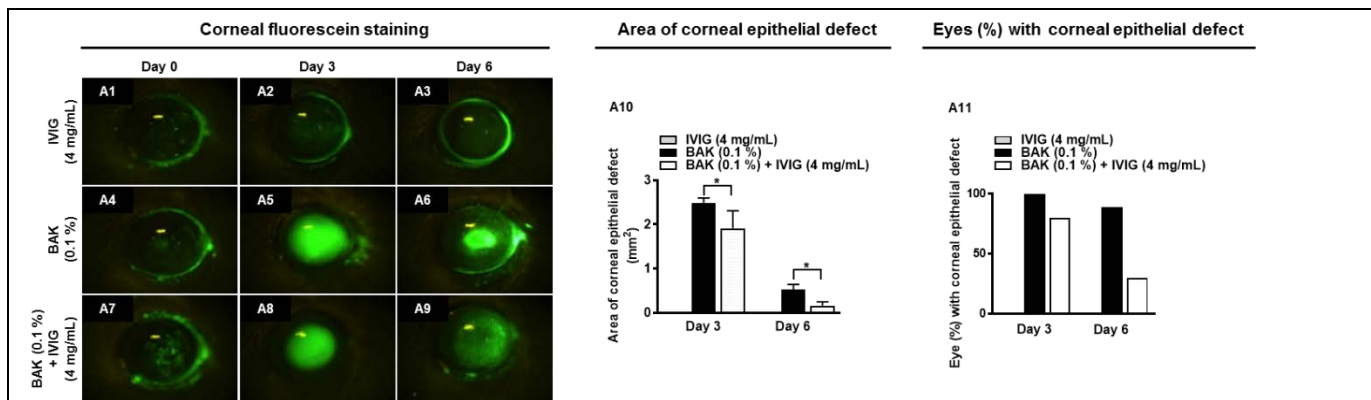


Figure 11: *In vivo* efficacy study of IVIG-eye drop to treat corneal inflammation induced by Benzalkonium chloride (BAK).

*These experiments were performed to determine whether IVIG-eye drop (4 mg/mL) reduces ocular surface inflammation in murine corneas. In the control groups, IVIG (4 mg/mL) only or BAK (0.1%) only were applied to murine corneas (n=6/group) once a day for 6 consecutive days to induce corneal inflammation. In the experimental group, BAK (0.1%) and IVIG (4 mg/mL) were applied to the murine corneas (n=6) once a day for 6 consecutive days. After each application, the mice were restrained for 1 minute to allow for adequate contact time of eye drop with the eye. Fluorescein staining was performed at base line and every other day. (A1-A3): Representative mouse cornea images showing no increased in fluorescein staining in control group IVIG (4 mg/mL) only. Compared to BAK only group (A4-A6), IVIG and BAK group (A7-A9) showed significantly less corneal staining at Day 3 and Day 6. Corneas in BAK only group and IVIG + BAK group had epithelial defects. We compared the area of epithelial defect (A10) and the number of eyes (%) that had corneal epithelial defect (A11) between BAK only group and IVIG + BAK group. In the IVIG + BAK group, the corneal epithelial defect area was significantly less than in BAK only group (A10) and the percentage of eyes with corneal epithelial defect was also significantly less (A11). ***Taken together, these data show that IVIG application to the eye reduces corneal inflammation.****

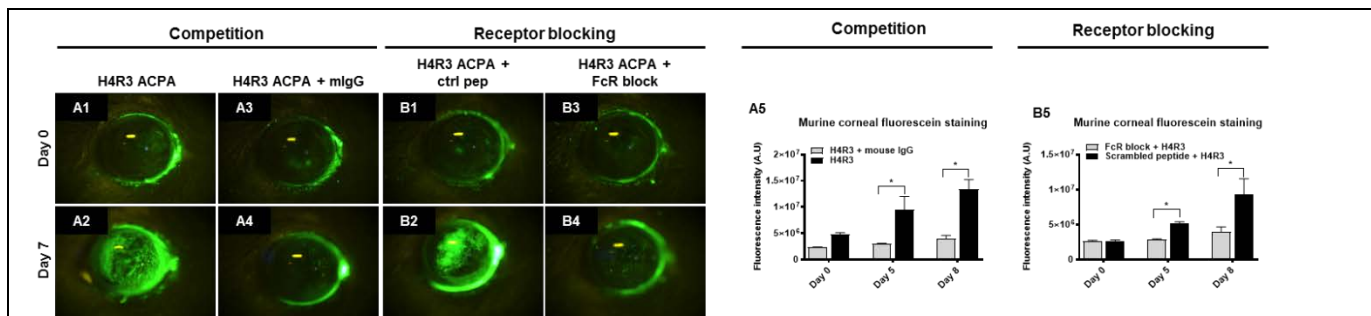


Figure 12: *In vivo* efficacy study to determine the therapeutic potential of blocking the interaction of Anti-Citrullinated Protein Antibody (ACPA) with Fc receptors on murine corneas.

These experiments were performed to determine whether strategies to block ACPA-Fc receptor interaction (using either IgG for competitive blocking or peptides for Fc receptor blocking) abrogate ACPA-induced ocular surface disease. Citrullinated Histone H4 antibody (H4R3 ACPA, 100 ng/mL) was applied to the mouse cornea to produce ocular surface disease. Mouse IgG (100 ng/mL) was used for competitive blocking (Experiment 1) and a peptide (1:40 dilution) was used for Fc receptor blocking (Experiment 2). Experiment 1: In control group, H4R3 ACPA (10 uL solution) only was applied over the ocular surface of anesthetized mice for 40 minutes once a day for 7-8 consecutive days. In experimental group, H4R3 ACPA + mouse IgG was applied in identical amount and time as the control group. Ocular surface disease was assessed with fluorescein staining. Representative mouse cornea images showing fluorescein staining after incubation with H4R3 ACPA solution only (A1-A2) and H4R3 ACPA + mouse IgG solution (A3-A4). Fluorescein staining of corneas in H4R3 ACPA + mouse IgG group was significantly lower than H4R3 ACPA group alone (A5). Experiment 2: In control group, a scrambled peptide (10 uL) was applied to the eye of an unanesthetized mice. After the application, the mice were restrained for 1 minute to allow for adequate contact time of eye drop with the eye. 30 minutes after control peptide application, H4R3 ACPA (10 uL solution) was applied over the ocular surface of anesthetized mice for 40 minutes. In the experimental group, azide-free Fc receptor blocker (Innovex Biosciences # NB355) (10 uL) and H4R3 ACPA were applied to mouse eye in a manner identical to the control group. Representative mouse cornea images showing fluorescein staining after incubation with control peptide + H4R3 ACPA (B1-B2) and Fc receptor blocking peptide + H4R3 ACPA (B3-B4). Fluorescein staining of corneas in Fc receptor blocking peptide + H4R3 ACPA group was significantly lower than control peptide + H4R3 ACPA (B5). **Taken together, these experiments show that blocking the interaction of ACPA with Fc receptors reduces ocular surface disease. Therefore, using IVIG eye drops may have therapeutic potential via this mechanism.**

1.11.2 IVIG Clinical Data to Date

Although clinical data regarding IVIG eye drop use in ocular surface disease does not exist, sufficient clinical data is available regarding systemic administration of IVIG for treating ocular surface diseases. IVIG therapy (given via intravenous route) has been used to treat Mucous Membrane Pemphigoid (MMP), a severe ocular surface disease, in patients who do not respond to conventional therapy or stopped using them for various side effects. A review of literature identified 13 studies with a total number of 70 patients with MMP who responded favorably with IVIG therapy.²⁸ Adverse effects associated with IVIG therapy were considerably lower than conventional therapy. We have measured tear fluid levels of IVIG after systemic administration for treating ocular MMP or ocular oGVHD. After systemic IVIG administration levels of IVIG are significantly higher in the tear fluid. This implies that the ocular surface is exposed to IVIG with systemic administration because IVIG is present in the tear fluid. Our hypothesis is that applying IVIG to the ocular surface as eye drops maybe a safer, more cost

effective and potentially more efficacious method of IVIG treatment of ocular surface disease as compared to systemic administration. Because IVIG is present in the tears with systemic administration and no ocular side effects have been reported, we expect IVIG-eye drops to have a similar safety profile.

Immunoglobulins (IgG) have been placed on the ocular surface in the form of autologous/allogeneic serum/plasma eye drops for treating ocular surface diseases. Blood derived products (unfractionated serum or plasma eye drops) are essentially physiological IgG formulations, therefore clinical data pertaining to their use in treating ocular surface diseases is relevant to the proposed fractionated plasma product (IVIG eye drops). This is because the IgG concentration in unfractionated serum/plasma eye drops (50%) and the proposed IVIG eye drops is similar (4 mg/ml). Normal average serum IgG values are 10 mg/ml.^{11, 29} Serum also contains important nutritive elements (such as vitamin A, epidermal growth factor, transforming growth factor- β , fibronectin, nerve growth factor and insulin-like growth factor) that promote the viability of ocular surface cells.³⁰⁻³⁴ Serum can be formulated as autologous or allogeneic serum tear eye drops in specialized laboratories or compounding pharmacies. Autologous serum drops are produced from the patient's own peripheral blood, whereas Allogeneic serum drops made from voluntary donors.^{35, 36}

Autologous Serum eye drops have been used for the treatment of ocular surface disorders, including dry eye, neurotrophic keratitis, exposure keratitis, graft-versus-host disease, Sjögren syndrome, and mucous membrane pemphigoid.³⁷⁻⁴⁹ Controlled clinical trials have shown the superiority of autologous serum eye drops compared with artificial tears when used to treat some of these conditions.^{43, 50, 51} Our research findings lead us to posit that IgG in serum contributes to its beneficial effects in autoimmune and inflammatory ocular surface diseases.

Although autologous serum is more commonly used, **Allogeneic serum eye drops** made from voluntary donors⁵² have been used in Denmark,⁵³ Norway,⁵⁴ and the Netherlands^{55, 56} as a means of minimizing the hazards and costs associated with the preparation of an autologous product. Allogeneic serum may be an appropriate alternative for selected patients who would benefit from serum treatment but whose autologous serum is unavailable or unsuitable.^{52, 57-60}

Limitations of Serum eye drops: One important practical limitation in treatment with serum eye drops is the very short shelf life. The United States Pharmacopeia standard recommends a 45 day expiration date for the prescription while it is stored in the freezer. Once a bottle is thawed for use, they have to be kept refrigerated at 4 C and they expire after 3 days. Other practical issues are that only a compounding pharmacy can prepare the eye drops and serum eye drops lack insurance coverage.

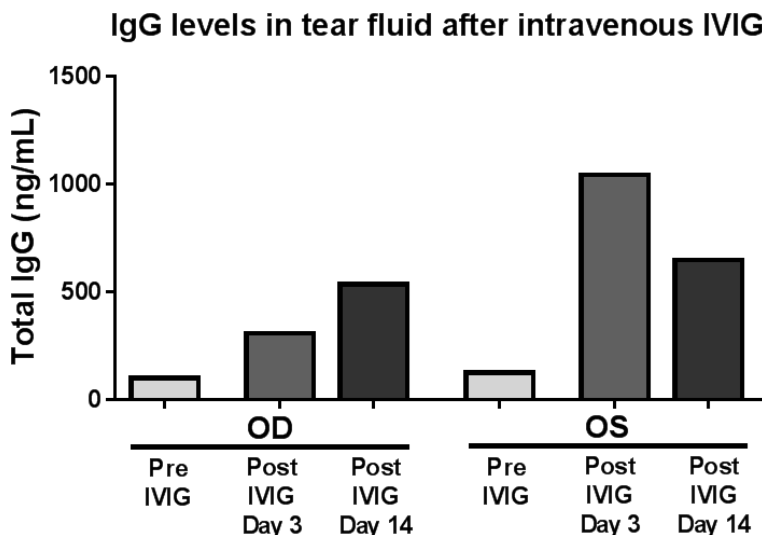


Figure 13: IgG levels in tear fluid after intravenous administration of IVIG to treat DED in a patient with ocular cicatricial pemphigoid (OCP). We determined whether IgG levels in tear fluid increase after IVIG is administered by the intravenous route. A 39 year old male had severe symptoms of ocular discomfort and severe tear deficient DED due to OCP. A decision was made to treat him with intravenous IVIG. Flebogamma 5% 60g/1200 mL IV per protocol was administered. Tear fluid was collected prior to IVIG infusion and following IVIG infusion and analyzed for total amount of IgG using Human Immunoglobulin Isotyping MILLIPLEX MAP kit (Cat. No. HGAMMAG-301K, EMD Millipore, Billerica, MA).. At day 3 post IVIG infusion, the IgG level in tear fluid showed 2.8 fold increase in right eye and 7.5 fold increase in left eye. At day 14 post IVIG infusion, IgG level in tear fluid showed 4.8 fold increase in right eye and 4.7 fold increase in left eye. Taken together, these data showed that IgG levels in tear fluid increases several fold after intravenous IVIG administration.

1.12 Dose Rationale and Risk/Benefits

Proposed dose: IVIG eye drops 4 mg/ml (0.4%) two times a day.

Rationale: Serum eye drops, compounded by diluting serum by 50% in normal saline, are routinely used for treating ocular surface diseases. The amount of IgG in undiluted serum is approximately 10 mg/ml, therefore 50% serum eye drops has approximately 5 mg/ml IgG, and serum eye drops are generally used four times a day. Since, at 50% dilution serum eye drops are effective in treating ocular surface diseases, we have proposed a similar dose of IgG in IVIG eye drops (4 mg/ml). In our in vitro experiments, we have determined 4 mg/ml IVIG to be non-toxic, as this dose, when added to medium of cultured epithelial cells for 24 hours, did not cause an increase in LDH concentration in the supernatant.

Also, it is generally accepted that IVIG should be given at a concentration that is at least 4 times the concentration of IgG at the application site. In ocular surface diseases, we have estimated IgG amount on the ocular surface to be approximately 0.5 mg/ml, therefore the IVIG dose should be at least 2 mg/ml to be effective. Taken together, our proposed dose of IVIG eye drops replicates the clinically in-use dose of IgG in serum tears, is non-toxic in vitro, and above the threshold required to be effective.

Adverse Effects of IVIG Treatment: To date, IVIG has been administered via systemic route only. Mild to moderate adverse reactions to high-dose IVIG therapy might be seen in nearly 30% of patients. Some of the common adverse reactions include fever, headache, chills, nausea, hypotension, and muscle cramps. Temporary cessation of the IVIG infusion, slow infusion, and use of general anti-inflammatory agents can control these events. Switching to SCIG is another viable alternative. Anaphylaxis can occur in IgA-deficient patients and use of low IgA-containing IVIG preparations is recommended in such cases. Other effects include meningeal inflammation and aseptic meningitis due to the release of inflammatory cytokines and neutrophil activation by IgG Abs within IVIG that mimic antineutrophil cytoplasmic Abs; intravascular hemolysis due to the presence of anti-A or anti-B isoagglutinins or less commonly anti-D or anti-K Abs; thromboembolism because of contamination of IVIG with clotting factors and formation of platelet-leukocyte aggregates; and renal complications due to osmotic injury that could be prevented by using non-sugarstabilized IVIG.

Possible contamination of IVIG with infectious agents like viruses and prions always exists. However, viral inactivation steps, caprylation, and nanofiltration aid in the safety profile of IVIG.

2 Study Objectives

The main objective of this study is to establish whether patients with Dry Eye Disease are able to safely tolerate receiving Intravenous Immunoglobulin (IVIG) eye drops two times a day for eight weeks (primary 'safety and tolerability' objective). The exploratory objective is to investigate the preliminary efficacy of the use of IVIG eye drops in treating Dry Eye Disease (exploratory efficacy objective) to estimate the effectiveness of the trial intervention and collecting data to inform the design of a future definitive trial.

3 Study Design

3.1 General Design

This will be a Randomized controlled trial, in which a total of 28 subjects will be enrolled at 1 clinical site. Subjects will be randomly assigned to one of two groups (#1, #2), with 14 subjects per group. One group will be given placebo (Normal saline eye drops) and the other group will be given eye drops containing the study drug (IVIG).

Patients with established Dry Eye Disease 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 Dry Eye Disease. 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 at 4 weeks for further study assessments,

thereafter at 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.

There is no primary efficacy endpoint in this study. Instead several exploratory efficacy endpoints will be assessed.

The primary tolerability endpoint is the change in the test substance tolerance between Day 1 (post-dose) and 8 weeks (56 days).

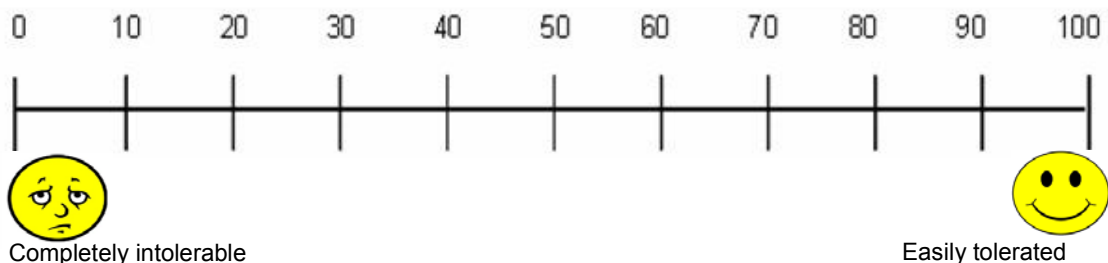
3.2 Tolerability End Point: Test Substance Tolerance (Visual Analogue Scale)

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 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 4 and week 8. Subjects will place a single slash mark across the horizontal line between the end labeled “completely intolerable” (0 mm) and “easily tolerable” (100mm). The VAS rating is as follows:

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

Visual Analogue Scale

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



3.3 Secondary Study Endpoint

There are no secondary endpoints in this study.

3.4 Exploratory Study Endpoints

The exploratory 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 corneal staining score as measured by Lissamine dye staining

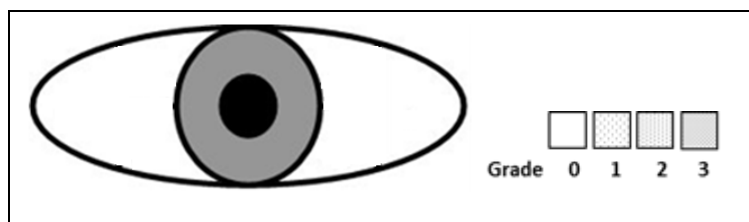
5. The change in subjective ocular surface redness score (OR) using the validated bulbar redness (VBR) grading scale
6. Visual acuity change
7. Change in frequency of administration of artificial tears or concomitant eye drops
8. Change in number of corneal filaments (slit-lamp examination)
9. Change 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 (NIK BUT)
13. Change in Keratograph Ocular Bulbar Redness Score
14. Change in MMP-9 Protein Detection
15. Change in amount of Cytokines in ocular surface wash

3.4.1 Exploratory Efficacy End Point: Ocular surface Lissamine Dye staining score

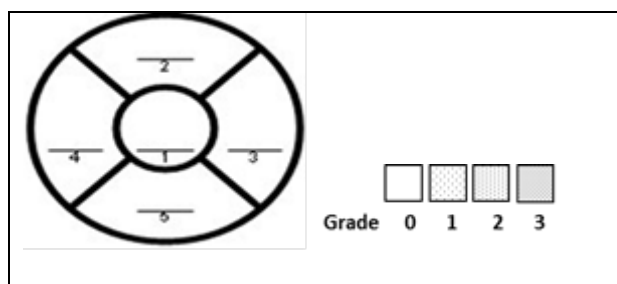
Ocular surface staining will be assessed using Lissamine Green Dye for Cornea and Conjunctiva. The scoring pattern is represented below.

Lissamine Green Dye Staining for Cornea and Conjunctiva:

Using 5uL fixed pipette and a 10uL pipette tip 5uL of the Lissamine will be drawn from the Eppendorf vial and after slightly pulling the eye lid of the patient Lissamine dye will be released over the ocular surface of both eyes. Using the slit lamp nasal-bulbar and temporal-bulbar conjunctiva will be graded within 2 minutes for each eye.



Corneal staining will be graded in 5 zones. Each zone will be graded from 0 to 3 based on the density of punctate staining.



3.4.2 Exploratory 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 4, week 8 and week 10. The questions and scoring system are shown below:⁶¹

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

3.4.3 Exploratory 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 as follows: ⁶²

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

3.4.4 Exploratory 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:⁶²

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?

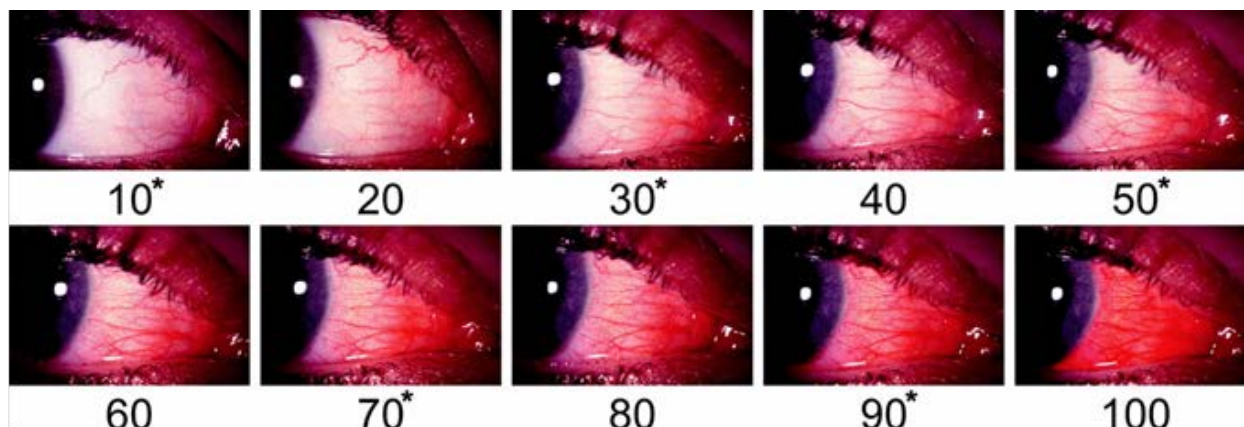
The responses will be categorized on a five point scale as follows:

Much worse
Worse
About the same
Improved
Much improved

3.4.5 Exploratory Efficacy End Point: Ocular surface redness score

Ocular surface redness (nasal or temporal) will be assessed using the Validated Bulbar Redness grading scale (VBR).⁶³ 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 and the bulbar conjunctival injection of the subject's eye (nasal and temporal) will be compared to the reference images and graded accordingly.

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

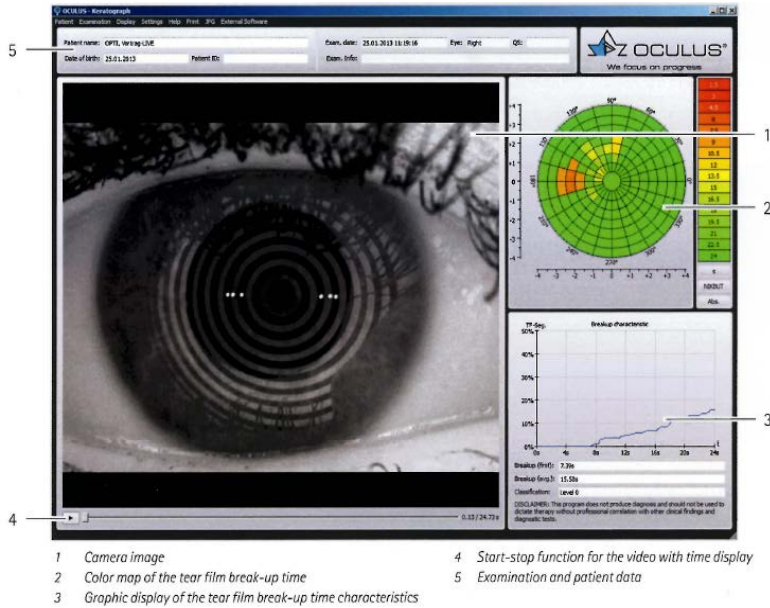


3.4.6 Exploratory Efficacy End Point: Keratograph Oculus Redness Score

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 (NIK BUT)

The non-invasive Keratograph tear film break-up time (NIK BUT) measures tear film stability. The NIK BUT 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 (NIK BUT) tool. Based on the device IR video, the device generates 2 measures for TBUT: NIK BUT-first (time at which the first breakup of tears occurs) and NIK BUT-average (average time of all breakup incidents) automatically and without touching the eye.



3.4.7 Exploratory 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. The test is assembled by gently placing the sampling fleece of the sample collector into the sample transfer window of the test cassette body. The buffer vial is opened and immersed in the absorbent tip. The absorbent tip is removed from the buffer vial, the protective cap is replaced, and the test is laid flat on a horizontal surface. 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-immerses 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.



3.4.8 Exploratory Efficacy End Point: Symptom (Ocular Discomfort) Intensity

Symptom (ocular discomfort) intensity will be assessed utilizing a Visual Analog Scale (VAS). The VAS is a horizontal line with verbal descriptor anchors at regular intervals. Symptom intensity will be

assessed on baseline, week 4, week 8, and week 10. Subjects will be asked to assess their symptom intensity as a fraction of 10. The symptom intensity will be assessed as follows:

Question to the patient: On a scale from 0-10, what was the intensity of your ocular discomfort, at its worst, over the past 24 hours?



3.5 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.

In addition to the primary tolerability endpoint (Section 2.2), the following safety endpoints will be assessed:

- The proportion of screened subjects who are successfully recruited in the study (i.e., recruitment rate)
- The proportion of eligible subjects at Day 56 who successfully complete (i.e., retention rate, (non)compliance or adherence rate) a full eight weeks (i.e., 56 days) of therapy with topical administration two times per day (BID).
- All reported adverse events, classified by frequency, severity, and relatedness, from baseline (Day 1) through the last study visit (Day 70).
- Clinically significant changes in vital signs or ophthalmic examination from baseline.

3.5.1 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 week 4, 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 (⁰F). Subjects with an oral temperature less than (\leq) 99.6⁰F (37.4⁰C) may continue.

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

3.5.2 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 (if they are present) 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 3.4.5. Measurements at first and last visit will include: visual acuity (Snellen's chart), manifest refraction and intraocular pressure measurement. Clinically significant changes from baseline examination will be recorded on the adverse event forms. 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).

3.6 Other Study procedures: Ocular Surface Washings

At the first (Baseline) and last treatment (8 week) visits, ocular surface washings will be collected as follows: 50 μ L of artificial tear (Preservative Free Refresh Optive Sensitive, Allergan, Irvine, CA) was instilled into the lower fornix of the eye. The patient will be instructed to perform ductions in all directions, and after approximately 1 minute the conjunctival washings were collected with a 5 μ L glass microcapillary tube (Microcaps Drummond Scientific, Broomall, PA) and transferred to a sterile 0.2 mL microcentrifuge tube. The samples will be stored at cold temperature (approximately 4 degrees C) until further analysis. Ocular Surface washings will be transported to the laboratory and used for potential biomarker analysis etc.

4 Subject Selection and Withdrawal

4.1 Inclusion Criteria

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

1. Sign and date the informed consent form approved by the IRB
2. ≥ 18 years of age
3. Demonstrate at least any 2 of the following signs in the same eye or a sign and symptom.
 - a) Conjunctival staining present ≥ 1 (out of possible score of 6 per eye)
 - b) Corneal staining present ≥ 2 (out of a possible score of 15 per eye)
 - c) Tear film break up time (TFBUT) ≤ 7 seconds
 - d) Schirmer's test ≥ 0 to ≤ 9 mm/5min
 - e) SLK pattern staining ≥ 1
 - f) Meiboscale grade ≥ 2
 - g) Validated Bulbar Redness ≥ 40
 - h) Demonstrate symptoms of dry eye disease OSDI score of at least ≥ 13 .
 - i) Demonstrate Symptom Intensity Assessment of ≥ 3 .
4. Patient reported dry eye-related ocular symptoms for at least 6 months before the Screening Visit and use or desire to use artificial tears on average 2 times per day in the 2 weeks preceding the screening visit
5. Intraocular pressure (IOP) ≥ 5 mmHg and ≤ 22 mmHg in each eye
6. Women of child-bearing potential must agree to use a reliable method of contraception during study participation and must demonstrate a negative pregnancy test at the Screening Visit
7. Be willing/able to return for all study visits and to follow instructions from the study investigator and his/her staff
8. Ocular Surface Wash Anti-Citrullinated Protein Antibody (ACPA) value of > 4.4 units in either eye at any time in the past.

4.2 Exclusion Criteria

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

1. Allergic to IVIG or any similar products, or excipients of IVIG eye drops 4 mg/ml.
2. Use of contact lenses within the last 2-weeks prior to the baseline Visit.
3. Use of Allogenic serum or plasma eye drops within the last 2-weeks prior to the baseline Visit.
4. Unwilling to commit to no use of contact lenses for the duration of the study.
5. Pregnant or nursing/lactating
6. Participation in a study of an investigational drug or device within the 30 days preceding the Screening Visit
7. Current diagnosis of any of the following ocular conditions:
 - i) Acute allergic conjunctivitis
 - ii) Active infection (e.g. bacterial, viral, protozoan or fungal infection of the cornea, conjunctiva, lacrimal gland, lacrimal sac or eyelids)
 - iii) Active intraocular inflammation (e.g., retinitis, choroiditis, uveitis)
8. Ocular surgery (including cataract surgery), by patient report, within 3 months of Screening Visit
9. Previous LASIK surgery or any other corneal transplant surgery, by patient report.
10. Cognitive or psychiatric deficit that precludes informed consent or ability to perform
11. Vulnerable populations, such as neonates, pregnant women, children, prisoners, institutionalized individuals, or others who may be considered vulnerable populations.
12. Have active drug/alcohol dependence or abuse.
13. Corneal epithelial defect larger than 1 mm² in either eye.
14. Active ocular infection or ocular allergies.

Participants will be permitted to continue their chronic ocular treatments, including the use of artificial tears, ointments, prescription eye drops (Restasis, Xiidra, Steroids), eyelid massage, or warm compresses. Subjects will be asked to maintain their ocular treatments unchanged in frequency of use for the duration of the study. Subjects wearing contact lenses (Soft bandage contact lenses or PROSE lens) or using serum tears will be asked to discontinue contact lens wear or use of serum tears for 2-weeks prior to being enrolled (Baseline visit) and will be required to not wear contact lenses or use of serum tears for the duration of the study (until week 10 visit). All systemic medications will continue unchanged.

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 DED in the investigator's eye clinic (cornea clinic or the comprehensive eye clinic), in the Illinois Eye and Ear Infirmary. Furthermore, these patients would include only those who have had an ACPA value of > 4.4 units in ocular surface washings of either eye at any time in the past. Patients diagnosed with DED, 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, RUSH University, Cook County Hospital, 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 screened, recruited, and will attend all study related visits only at the PI's clinic in Illinois Eye and Ear Infirmary.

Patients with Dry Eye Disease (with Schirmer's test ≥ 0 to ≤ 9 mm/5min 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 performing any screening tests to determine eligibility. Screening procedures include documentation of DED 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, IVIG-eye drops, will be compounded in the UIC Eye and Ear Infirmary (EEI) Pharmacy and supplied as a 4 mg/ml (0.4%) preservative free solution in single use vials for administration as topical eye drops. The control group will receive Normal saline eye drops as placebo.

Each subject will receive either IVIG-eye drops (study drug) or Normal saline (placebo) solution, as a single eye drop in each eye two times a day (BID) 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- IVIG-eye drops, 4mg/ml eye drops will be applied to both eyes b.i.d for 8 weeks. Control group – Normal saline eye drops will be applied to both eyes b.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 other dose at approximately 12 hour interval. Therefore, doses will be scheduled at approximately 8 a.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 (IVIg 4mg/ml; test group), and the other group will receive placebo (Normal saline; control group). We will use a computer-based random code generator (Research Randomizer; <http://randomizer.org/>) to generate 1 set of 24 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, e.g. Subject #1, subject #2, and 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. 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, except dilution with preservative free normal saline to achieve 4mg/ml concentration, will be made to the study medication constituents. The drug and placebo will be dispensed in sterile eye droppers as single use vials, as explained below:

The study medications will be dispensed in sterile eye droppers of 3.0 ml volume. The commercially available product (Flebogamma or an equivalent IVIG preparation) is a single-use ampule containing 5% (50mg/ml) concentration of IVIG. Flebogamma (5%) will be diluted in normal saline to achieve IVIG-eye drops 0.4% (4mg/ml). 0.5 ml to 1.0 ml of IVIG-eye drop (4mg/ml) will be transferred into one sterile eye droppers of 3.0 ml volume. IVIG-eye drops preparation (by diluting Flebogamma) 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, 2 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 for 28 days.** Prepared eye droppers will be placed in a dark (brown) colored zip-lock packet before being dispensed to the subject. The medication can be stored at room temp, 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 4, 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 Dry Eye Disease. 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) or using serum tears, they will be asked to discontinue contact lens wear or use of serum tears for 2-weeks prior to being enrolled (Baseline visit) and will be required to not wear contact lenses or use serum tears for the duration of the study (until week 10 visit). This is necessary as one of the proposed exploratory study outcome measure is corneal Staining. Contact lenses can directly reduce corneal epitheliopathy and staining due to ensconcing and acting like a bandage that prevents corneal exposure to tear fluid inflammatory materials. Thus, because of overlapping effects on the exploratory outcome measure, it may not be possible to attribute any observed clinical benefit to IVIG-eye drops 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').⁶⁴ Stopping contact lens wear may increase the risk of worsening of Dry Eye Disease (DED) 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.

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 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.

The subjects will be monitored at 4 week intervals to ensure that they are not subjected to any undue risks during the course of the study. Additionally, they will be warned of the possible signs and symptoms of clinical worsening of DED, and advised to contact the research team immediately in case any of those symptoms occur. Subjects will also be encouraged to contact the research team in case they experience any ocular discomfort during the wash out period or during the course of the study. The subject's condition will be monitored by the physician (Principal Investigator) at each study visit, as well as at any interim visit (in case of adverse symptoms, as mentioned above). Any worsening of DED or any adverse event due to the study drug will be recorded. In case of clinical worsening, based on the individual subject's clinical condition, one or more of the following therapeutic decisions may be implemented: (1) Increasing/adding the use of artificial tears or ointments (2) Increasing/adding the use of anti-inflammatory therapy (Restasis/ Xiidra/ Steroids) or Serum Tears, (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 upon 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. Subjects will be withdrawn from the study for presumed ocular allergic reaction to the product and ocular infection. The study will be stopped if the incidences of presumed ocular allergic reaction to the product and ocular infection surpasses 20% for each.

5.7 Rescue Plan

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

- (1) Increasing/adding the use of artificial tears or ointments,
- (2) Increasing/adding the use of anti-inflammatory therapy (Restasis/ Xiidra/ Steroids) or Serum Tears
- (3) Discontinue the study drug.
- (4) Other eye drops/measures (example, contact lens or surgeries), if indicated.

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 reinstated 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 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 2 eye droppers will be required per day because the placebo/drug is administered two times a day. At each visit, subjects will receive 56 sterile 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 4 weeks of remaining doses in a dark packet to take home. 56 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) or EEI Pharmacy 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 at room temp, away from direct strong light.

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 4 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 4 week supply will be dispensed by the pharmacy. This will be done at first treatment visit (day 1) and week 4. No new drug eye droppers will be given on the 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 principal investigator (PI) 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. Subjects will include patients who have been diagnosed with dry eye in the investigator's eye clinic (cornea clinic or the comprehensive eye clinic), in the Illinois Eye and Ear Infirmary. Patients may also be referred to the PI's clinic by other Ophthalmology physicians at UIC, as well as the Ophthalmology departments at Loyola University Chicago, University of Chicago, RUSH University, Cook County Hospital, and Northwestern University. These centers will be provided with 'information sheets' to provide to subjects before they come to the PI's clinic. The referring physicians shall inform the patients briefly about the study and provide them with the information sheet (enclosed), but no study related procedures (including screening and recruitment) will be performed at any location outside the PI's clinic at UIC (Illinois Eye and Ear Infirmary). Even the patients referred to the PI's clinic for possible enrollment in the study, will be screened and, if eligible, recruited in the study only at the PI's clinic. The information sheet that will be given to the subjects are attached.

Patients with established Dry Eye Disease (for at least 6 months, with Schirmer's test ≥ 0 to ≤ 9 mm/5min and annoying or activity limiting visual symptoms) will be approached by a member of the research staff to determine if the patient might be interested in participating in a research study. If the 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 password protected 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 post publication of the study results 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) 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 -21 to 0

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

- Demographic information including: birth date, gender, race or ethnic origin.
- Medical History including prior medication use and prior procedures: Medical history will be obtained by interviewing the subject and will include a review of the following systems: cardiovascular, dermatologic, gastrointestinal, genitourinary, musculoskeletal, neurologic and respiratory. An allergic history (including medications and food), substance abuse history (including alcohol) and a history of medication use (including prescription, OTC, and herbal products) during the past 30 days will also be completed.
- Ophthalmic and Dry Eye Disease history including: date when the Dry Eye Disease began, verification that the subject has had Dry Eye Disease for at least 6 months, medications used by the subject to treat Dry Eye Disease, previous procedures to treat Dry Eye Disease.

- Ophthalmic Examination (slit lamp examination, Ocular Surface Redness Score, Ocular Surface Disease Index, Lissamine Dye staining, and a Schirmer I test)
- Pregnancy test, 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 DED 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) or using serum tears, they will be asked to discontinue contact lens wear or use of serum tears for 2-weeks prior to being enrolled (Baseline visit) and will be required to not wear contact lenses or use serum tears for the duration of the study (until week 10 visit). The study doctor will provide advice about decreasing DED symptoms with artificial tears, eyelid massage, or warm compresses.

If the subject agrees to enter the study after screening, the “Visit 2 Day 1 (Randomization and First treatment visit)” will be scheduled. Visit 2 can be scheduled any time in the next 2 weeks after the screening visit. If a subject wears contact lenses or uses serum tears and wants to participate in the study, then they will be asked to discontinue the use of contact lenses or use of serum tears and a washout period of 2-weeks (± 2 days) will be needed before baseline visit (Visit 2).

In some subjects, detailed screening may not have to be performed as the subjects may have been followed and treated for Dry Eye in the PI’s clinic. In these cases, some or all of the screening test may have been performed during the subject’s routine dry eye care. These subjects will not be screened formally. An informed consent will be administered and subjects will be given an appointment for baseline visit examination.

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

Prior to first dose (Baseline)

- Record concomitant medications
 - Vital Signs: Blood pressure, Pulse, and Temperature taken while subject is in a sitting position.
 - Visual Acuity (Snellen’s chart) with eye glasses (if applicable)
 - OSDI
 - Symptom Intensity Assessment
 - Keratograph Oculus Redness Score
 - > Non-invasive Keratography Tear Film Break-up Time (NIK BUT)
 - Schirmer I test
 - Baseline Ophthalmic Examination (slit lamp examination, VBR)
 - Ocular surface wash collection for analysis (Potential biomarkers)
 - InflammADry Test for MMP-9 Protein
 - Lissamine Dye Corneal and Conjunctival staining
 - Adverse events since screening visit.
- ***Randomization (by pharmacy)***
 - ***Dispensing of study drug/placebo (by Pharmacy)***
 - ***Investigator/designee administers first dose of study drug/ placebo***

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 4 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.
- Intraocular pressure measurement after topical anesthesia to eye

6.5.3 Visit 3: Week 4 (\pm 4 days)

Treatment Visit (after any dose for that day)

- Review the subject's diary and record changes in concomitant medication, deviations from drug schedule and adverse events.
- Vital Signs: Blood pressure, Pulse, and Temperature taken while subject is in a sitting position.
- Visual Acuity (Snellen's chart) with eye glasses (if applicable)
- OSDI
- Symptom Intensity Assessment, SGA and CGA
- Test Substance Tolerance (VAS)
- Baseline Ophthalmic Examination (slit lamp examination, VBR)
- *Dispensing of study drug/placebo (by Pharmacy to patient)*
- *Returning of unused study drug/ placebo (by patient to pharmacy)*

6.5.4 Visit 4: Week 8 (\pm 4 days)

Treatment Visit (after any dose for that day)

- Review the subject's diary and record changes in concomitant medication, deviations from drug schedule and adverse events.
- Vital Signs: Blood pressure, Pulse, and Temperature taken while subject is in a sitting position.
- Visual Acuity (Snellen's chart) with eye glasses (if applicable)
- OSDI
- Symptom Intensity Assessment, SGA and CGA
- Test Substance Tolerance (VAS)
- Keratograph Oculus Redness Score
- > Non-invasive Keratography Tear Film Break-up Time (NIK BUT)
- Schirmer I test
- Baseline Ophthalmic Examination (slit lamp examination, VBR)
- Ocular surface wash collection for analysis (Potential biomarkers)
- InflammDry Test for MMP-9 Protein
- Lissamine Dye Corneal and Conjunctival staining
- Intraocular pressure measurement after topical anesthesia to eye
- **Returning of unused study drug/ placebo (by patient to pharmacy)**

6.5.5 Visit 5: Week 10 (\pm 4 days)

Follow-Up Visit

- Review the subject's diary and record changes in concomitant medication and adverse events.
- Vital Signs: Blood pressure, Pulse, and Temperature taken while subject is in a sitting position.
- Visual Acuity (Snellen's chart) with eye glasses (if applicable)
- OSDI
- Symptom Intensity Assessment, SGA and CGA
- Baseline Ophthalmic Examination (slit lamp examination, VBR)

- **Returning of patient diary (by patient to PI)**

7 Statistical Plan

7.1 Sample Size Determination

As this is a feasibility trial intended to guide the planning of larger future investigations, there are no formal sample size calculations.⁶⁵ The aim of the study is to recruit a sufficient number of patients to evaluate the acceptability and safety of the intervention and the study design. An outcome of this study will be to estimate parameters such as the SD for a sample size calculation of a subsequent full-scale trial. A sample of 24 patients (12 in each arm) is considered adequate for obtaining reliable sample size estimates.^{66, 67} To allow for a conservative attrition rate of 10%, we aim to recruit 28 patients into the trial.

7.2 Statistical Methods

Based on the classification of reasons for conducting pilot studies by Thabane et al, in this pilot study we will assess the following: (i) Process– recruitment rate, retention rate, (non)compliance or adherence rate; and (ii) Scientific– safety (adverse event/SAE rate), tolerability, and efficacy, including reduction in clinical signs and symptoms (OSDI, Corneal staining, Redness etc as detailed in section 3.4) and treatment effect size. We will calculate the observed rate with 95% confidence interval for each rate by treatment group. Other descriptive statistics, including median with interquartile or mean with standard deviation for continuous variables and frequency with percentage for categorical variables will be reported as well. To estimate the preliminary efficacy under the scientific category, one eye (target eye) will be selected at screening visit 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 corneal stain score is used; (iii) if both eyes have the same corneal stain 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. Due to the nature of this feasibility study, any efficacy statistical tests and adjusted analyses will not be conducted. Detailed data validation will examine completeness, existence and accuracy of collected data to assess data quality and identify missing and conflicting data. Statistical analysis will be performed on an intention-to-treat basis i.e., inclusion of all patients randomly assigned, regardless of adherence, actual treatment received, and subsequent withdrawal of treatment and/or deviation from the protocol and per-protocol (i.e., inclusion of those who completed the treatment as planned) and will be reported according to 2010 CONSORT guidelines. All statistical analyses will be completed using SPSS Statistics V.22.0.31

7.3 Subject Population(s) for Analysis

Statistical analysis will be performed on: (i) intention-to-treat basis (i.e., inclusion of subjects randomly assigned, regardless of adherence, actual treatment received, and subsequent withdrawal of treatment

and/or deviation from the protocol); and (ii) per-protocol basis (i.e., inclusion of subjects who completed the treatment as planned).

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 Dry Eye Disease (DED) 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 IVIG, 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 IVIG; and/or the AE abates or resolves upon discontinuation of the IVIG or dose reduction and, if applicable, reappears upon re-challenge

No

Evidence exists that the AE has an etiology other than the IVIG (e.g., preexisting medical condition, underlying disease, intercurrent illness, or concomitant medication); and/or the AE has no plausible temporal relationship to IVIG 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 Adverse Event Reporting

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

8.6 Serious Adverse Event Reporting

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

1. Protocol Number
2. Initial reporter
3. Subject identification
4. Nature and date of the event/effect
5. Country of the event/effect
6. Severity of the event/effect
7. Reporting criteria
8. Narrative description of the event/effect
9. Outcome if known
10. Causal relationship to the investigational product
11. Additional and follow-up information as requested by the medical monitor.

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

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

8.7 In Case of an Emergency

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

8.8 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. Privacy, coding, storage: Research staff will be trained on the protocol requirements and data collection methods before completing study related procedures. Random study ID numbers will be assigned to study subjects. A master code list will link the subject MRN to the study ID number. The data collected will be stored on PI's desktop in a HIPPA compliant encrypted folder at the Ear and Eye Infirmary and Lions Eye Research building. The data collected, 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.

All Serious Adverse Events (SAE) that occur during the course of the study, and possibly related to the study intervention, including death, which are unanticipated will be reported to the IRB within 5 business days of the investigator becoming aware. A copy of the report will be sent to the FDA.

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

- Local adverse events or problems that are unanticipated and, while not meeting the criteria of serious, indicate research is associated with a greater risk of harm to participants or others than previously known.
- New information indicating an unexpected change to the risks or benefits of the research (i.e., an unanticipated problem).

A copy of this report will be sent to the FDA.

The minimum information to be provided includes:

1. Protocol Number
2. Initial reporter
3. Subject identification
4. Nature and date of the event/effect
5. Country of the event/effect
6. Severity of the event/effect

7. Reporting criteria
8. Narrative description of the event/effect
9. Outcome if known
10. Causal relationship to the investigational product
11. Additional and follow-up information as requested by the medical monitor.

8.9 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, including those with Meibomian Gland Disease. He routinely takes care of patients with severe ocular surface disease. He's also the director of Dry Eye service at UIC. Therefore, he's well qualified to recognize the symptoms and clinical signs of an adverse event. Dr. Jain has been the PI of past and active IRB approved clinical studies and has monitored data related to those studies. Therefore, he has experience in data and safety monitoring.

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

9 Confidentiality

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

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

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

10 Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions

certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

10.1 Records Retention

It is the investigator's responsibility to retain study essential documents for the full duration of the study. This includes, but is not limited to, the following: completed Informed Consent forms, Case Report Forms, AE/SAE report forms, all study correspondence and associated documentation. Per regulations, these records should be maintained for a period of two years following a marketing approval for the study drug or two years following shipment and delivery of the drug for investigational use is discontinued.

11 Ethical Considerations

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

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

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

12 Study Finances

12.1 Funding Source

- (i) UIC Office of Technology Management Proof of Concept Award
- (ii) Departmental funds

12.2 Conflict of Interest

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

12.3 Subject Stipends or Payments

The subjects will receive \$50 cash for baseline and week 8 visit and \$40 cash for all other completed study visits. If the subjects do not finish the study, they will be compensated for the visits they have

completed. The compensation will be provided as cash after each visit. If the subject completes the study, the total amount of compensation will be \$180.00.

13 References:

1. Fisher LD , Dixon DO , Herson J , et al . Intention to treat in clinical trials. Statistical issues in drug research and development. New York: Marcel Dekker, 1990:331–50.
2. Schulz KF, Altman DG, Moher D. CONSORT 2010 statement: updated guidelines for reporting parallel group randomized trials. *BMJ* 2010;340:c332.doi:10.1136/bmj.c332.
3. Craig JP, Nelson JD, Azar DT, Belmonte C, Bron AJ, Chauhan SK, de Paiva CS, Gomes JAP, Hammit KM, Jones L, Nichols JJ, Nichols KK, Novack GD, Stapleton FJ, Willcox MDP, Wolffsohn JS, Sullivan DA. TFOS DEWS II Report Executive Summary *Ocul Surf.* 2017 Oct;15(4):802-812.
4. Suvarna P. Phadatare, Munira Momin, Premanand Nighojkar, Sonali Askarkar, and Kamalinder K. Singh, “A Comprehensive Review on Dry Eye Disease: Diagnosis, Medical Management, Recent Developments, and Future Challenges,” *Advances in Pharmaceutics*, vol. 2015, Article ID 704946, 12 pages, 2015. <https://doi.org/10.1155/2015/704946>
5. Bron AJ, de Paiva CS, Chauhan SK, Bonini S, Gabison EE, Jain S, Knop E, Markoulli M, Ogawa Y, Perez V, Uchino Y, Yokoi N, Zoukhri D, Sullivan DA. TFOS DEWS II pathophysiology report *Ocul Surf.* 2017 Jul; 15(3):438-510
6. Messmer EM. The pathophysiology, diagnosis, and treatment of dry eye disease. *Dtsch Arztebl Int.* 2015;112(5):71-81; quiz 82.
7. Pflugfelder SC, de Paiva CS, Li DQ, Stern ME. Epithelial-immune cell interaction in dry eye. *Cornea.* 2008;27 Suppl 1(0 1):S9-11.
8. Stevenson W, Chauhan SK, Dana R. Dry eye disease: an immune-mediated ocular surface disorder. *Arch Ophthalmol.* 2012;130(1):90-100.
9. Dor M, Eperon S, Lalive PH, Guex-Crosier Y, Hamedani M, Salvisberg C, Turck N. Investigation of the global protein content from healthy human tears. *Exp Eye Res.* 2018 Oct 13;179:64-74
10. CHODIRKER WB, TOMASI TB Jr. GAMMA-GLOBULINS: QUANTITATIVE RELATIONSHIPS IN HUMAN SERUM AND NONVASCULAR FLUIDS. *Science.* 1963 Nov 22;142(3595):1080-1.
11. Agarwal S, Cunningham-Rundles C. Assessment and clinical interpretation of reduced IgG values. *Ann Allergy Asthma Immunol.* 2007 Sep;99(3):281-3.
12. Stuchell RN, Feldman JJ, Farris RL, Mandel ID. The effect of collection technique on tear composition. *Invest Ophthalmol Vis Sci.* 1984 Mar;25(3):374-7.
13. Knop N, Knop E. Conjunctiva-associated lymphoid tissue in the human eye. *Invest Ophthalmol Vis Sci.* 2000 May;41(6):1270-9.
14. Barisani-Asenbauer T, Inic-Kanada A, Belij S, Marinkovic E, Stojicevic I, Montanaro J, Stein E, Bintner N, Stojanovic M. The ocular conjunctiva as a mucosal immunization route: a profile of the immune response to the model antigen tetanus toxoid. *PLoS One.* 2013 Apr 26;8(4):e60682.
15. Hall JM, Pribnow JF. IgG and IgA antibody in tears of rabbits immunized by topical application of ovalbumin. *Invest Ophthalmol Vis Sci.* 1989 Jan;30(1):138-44.
16. Borderie VM1, Gineys R, Goldschmidt P, Batellier L, Laroche L, Chaumeil C. Association of anti-herpes simplex virus IgG in tears and serum with clinical presentation in patients with presumed herpetic simplex keratitis. *Cornea.* 2012 Nov;31(11):1251-6.
17. Skwor T, Kandel RP, Basravi S, Khan A, Sharma B, Dean D. Characterization of humoral immune responses to chlamydial HSP60, CPAF, and CT795 in inflammatory and severe trachoma. *Invest Ophthalmol Vis Sci.* 2010 Oct;51(10):5128-36.

18. Zandbelt M, te Boome L, Klasen I, van de Putte L, van den Hoogen F. Tear fluid measurement of anti-SS-A and anti-SS-B antibody in anti-SS-A and anti-SS-B seronegative Sjögren's syndrome patients. *Clin Exp Rheumatol*. 2009 May-Jun;27(3):536.
19. Amparo F, Schaumberg DA, Dana R. Comparison of Two Questionnaires for Dry Eye Symptom Assessment: The Ocular Surface Disease Index and the Symptom Assessment in Dry Eye. *Ophthalmology*. 2015;122(7):1498-503.
20. Saboo US, Amparo F, Abud TB, Schaumberg DA, Dana R. Vision-Related Quality of Life in Patients with Ocular Graft-versus-Host Disease. *Ophthalmology*. 2015;122(8):1669-74.
21. Lin H, Yiu SC. Dry eye disease: A review of diagnostic approaches and treatments. *Saudi J Ophthalmol*. 2014;28(3):173-81.
22. Martín-Nares E, Hernández-Molina G. Novel autoantibodies in Sjögren's syndrome: A comprehensive review. *Autoimmun Rev*. 2018 Dec 18. pii: S1568-9972(18)30292-1.
23. Dourmishev LA, Guleva DV, Miteva LG. Intravenous Immunoglobulins: Mode of Action and Indications in Autoimmune and Inflammatory Dermatoses. *Int J Inflam*. 2016;2016:3523057. PMID: 26885437
24. Tellier Z. Human immunoglobulins in intraocular inflammation. *Ann N Y Acad Sci*. 2007 Sep;1110:337-47.
25. LeHoang P, Cassoux N, George F, Kullmann N, Kazatchkine MD. Intravenous immunoglobulin (IVIg) for the treatment of birdshot retinochoroidopathy. *Ocul Immunol Inflamm*. 2000 Mar;8(1):49-57.
26. Rossi F, Kazatchkine MD. Antidiotypes against autoantibodies in pooled normal human polyspecific Ig. *J Immunol*. 1989 Dec 15;143(12):4104-9.
27. Rossi F, Dietrich G, Kazatchkine MD. Anti-idiotypes against autoantibodies in normal immunoglobulins: evidence for network regulation of human autoimmune responses. *Immunol Rev*. 1989 Aug;110:135-49.
28. Tavakolpour S1. The role of intravenous immunoglobulin in treatment of mucous membrane pemphigoid: A review of literature. *J Res Med Sci*. 2016 Jun 14;21:37.
29. Gonzalez-Quintela A1, Alende R, Gude F, Campos J, Rey J, Meijide LM, Fernandez-Merino C, Vidal C. Serum levels of immunoglobulins (IgG, IgA, IgM) in a general adult population and their relationship with alcohol consumption, smoking and common metabolic abnormalities. *Clin Exp Immunol*. 2008 Jan;151(1):42-50.
30. Liu L, Hartwig D, Harloff S, Herminghaus P, Wedel T, Kasper K, Geerling G. Corneal epitheliotropic capacity of three different blood-derived preparations. *Invest Ophthalmol Vis Sci*. 2006 Jun;47(6):2438-44.
31. Bradley JC, Bradley RH, McCartney DL, Mannis MJ. Serum growth factor analysis in dry eye syndrome. *Clin Exp Ophthalmol*. 2008 Nov;36(8):717-20.
32. van Setten GB, Viinikka L, Tervo T, Pesonen K, Tarkkanen A, Perheentupa J. Epidermal growth factor is a constant component of normal human tear fluid. *Graefes Arch Clin Exp Ophthalmol*. 1989;27(2):184-7.
33. Poon AC, Geerling G, Dart JK, Fraenkel GE, Daniels JT. Autologous serum eyedrops for dry eyes and epithelial defects: clinical and in vitro toxicity studies. *Br J Ophthalmol*. 2001 Oct;85(10):1188-97.
34. Tsubota K1, Goto E, Shimmura S, Shimazaki J. Treatment of persistent corneal epithelial defect by autologous serum application. *Ophthalmology*. 1999 Oct;106(10):1984-9.
35. Liu L, Hartwig D, Harloff S, Herminghaus P, Wedel T, Geerling G. An optimised protocol for the production of autologous serum eyedrops. *Graefes Arch Clin Exp Ophthalmol*. 2005 Jul;43(7):706-14.

36. Chiang CC, Chen WL, Lin JM, Tsai YY. Allogeneic serum eye drops for the treatment of persistent corneal epithelial defect. *Eye (Lond)*. 2009 Feb;23(2):290-3.
37. Matsumoto Y, Dogru M, Goto E, Ohashi Y, Kojima T, Ishida R, Tsubota K. Autologous serum application in the treatment of neurotrophic keratopathy. *Ophthalmology*. 2004 Jun;111(6):1115-20.
38. Ogawa Y, Okamoto S, Mori T, Yamada M, Mashima Y, Watanabe R, Kuwana M, Tsubota K, Ikeda Y, Oguchi Y. Autologous serum eye drops for the treatment of severe dry eye in patients with chronic graft-versus-host disease. *Bone Marrow Transplant*. 2003 Apr;31(7):579-83.
39. Tsubota K, Goto E, Fujita H, Ono M, Inoue H, Saito I, Shimmura S. Treatment of dry eye by autologous serum application in Sjögren's syndrome. *Br J Ophthalmol*. 1999 Apr;83(4):390-5.
40. Young AL, Cheng AC, Ng HK, Cheng LL, Leung GY, Lam DS. The use of autologous serum tears in persistent corneal epithelial defects. *Eye (Lond)*. 2004 Jun;18(6):609-14.
41. Geerling G, MacLennan S, Hartwig D. Autologous serum eye drops for ocular surface disorders. *Br J Ophthalmol*. 2004 Nov;88(11):1467-74.
42. Dogru M, Tsubota K. Pharmacotherapy of dry eye. *Expert Opin Pharmacother*. 2011 Feb;12(3):325-34.
43. Noble BA, Loh RS, MacLennan S, Pesudovs K, Reynolds A, Bridges LR, Burr J, Stewart O, Quereshi S. Comparison of autologous serum eye drops with conventional therapy in a randomised controlled crossover trial for ocular surface disease. *Br J Ophthalmol*. 2004 May;88(5):647-52.
44. Celebi AR1, Ulusoy C, Mirza GE. The efficacy of autologous serum eye drops for severe dry eye syndrome: a randomized double-blind crossover study. *Graefes Arch Clin Exp Ophthalmol*. 2014 Apr;252(4):619-26.
45. Jeng BH, Dupps WJ Jr. Autologous serum 50% eyedrops in the treatment of persistent corneal epithelial defects. *Cornea*. 2009 Dec;28(10):1104-8.
46. Alvarado Valero MC, Martínez Toldos JJ, Borrás Blasco J, Almiñana Almiñana A, Pérez Ramos JM. Treatment of persistent epithelial defects using autologous serum application. *Arch Soc Esp Oftalmol*. 2004 Nov;79(11):537-42.
47. Ferreira de Souza R1, Kruse FE, Seitz B. Autologous serum for otherwise therapy resistant corneal epithelial defects - Prospective report on the first 70 eyes. *Klin Monbl Augenheilkd*. 2001 Nov;218(11):720-6.
48. Hussain M, Shtein RM, Sugar A, Soong HK, Woodward MA, DeLoss K, Mian SI. Long-term use of autologous serum 50% eye drops for the treatment of dry eye disease. *Cornea*. 2014 Dec;33(12):1245-51.
49. Jirsova K, Brejchova K, Krabcova I, Filipec M, Al Fakih A, Palos M, Vesela V. The application of autologous serum eye drops in severe dry eye patients; subjective and objective parameters before and after treatment. *Curr Eye Res*. 2014 Jan;39(1):21-30.
50. Kojima T, Ishida R, Dogru M, Goto E, Matsumoto Y, Kaido M, Tsubota K. The effect of autologous serum eyedrops in the treatment of severe dry eye disease: a prospective randomized case-control study. *Am J Ophthalmol*. 2005 Feb;139(2):242-6.
51. Urzua CA, Vasquez DH, Huidobro A, Hernandez H, Alfaro J. Randomized double-blind clinical trial of autologous serum versus artificial tears in dry eye syndrome. *Curr Eye Res*. 2012 Aug;37(8):684-8.
52. Chiang CC, Chen WL, Lin JM, Tsai YY. Allogeneic serum eye drops for the treatment of persistent corneal epithelial defect. *Eye (Lond)*. 2009 Feb;23(2):290-3.

53. Harrithøj LH, Nielsen C, Ullum H, Hansen MB, Julian HO. Ready-made allogeneic ABO-specific serum eye drops: production from regular male blood donors, clinical routine, safety and efficacy. *Acta Ophthalmol.* 2014 Dec;92(8):783-6.
54. Espinosa A, Hjorth-Hansen H, Aasly K, Teigum I, Sivertsen G, Seghatchian J. Implementation of a standardised method for the production of allogeneic serum eye drops from regular blood donors in a Norwegian University Hospital: Some methodological aspects and clinical considerations. *Transfus Apher Sci.* 2015 Aug;53(1):88-91.
55. van der Meer PF1, Seghatchian J2, de Korte D2. Autologous and allogeneic serum eye drops. The Dutch perspective. *Transfus Apher Sci.* 2015 Aug;53(1):99-100. doi: 10.1016/j.transci.2015.05.017. Epub 2015 Jun 9.
56. van der Meer PF, Seghatchian J, Marks DC. Quality standards, safety and efficacy of blood-derived serum eye drops: A review. *Transfus Apher Sci.* 2016 Feb;54(1):164-7.
57. Chiang CC, Lin JM, Chen WL, Tsai YY. Allogeneic serum eye drops for the treatment of severe dry eye in patients with chronic graft-versus-host disease. *Cornea.* 2007 Aug;26(7):861-3.
58. Na KS, Kim MS. Allogeneic serum eye drops for the treatment of dry eye patients with chronic graft-versus-host disease. *J Ocul Pharmacol Ther.* 2012 Oct;28(5):479-83.
59. Stenwall PA, Bergström M, Seiron P, Sellberg F, Olsson T, Knutson F, Berglund D. Improving the anti-inflammatory effect of serum eye drops using allogeneic serum permissive for regulatory T cell induction. *Acta Ophthalmol.* 2015 Nov;93(7):654-7.
60. Marchand M, Harissi-Dagher M, Germain M, Thompson P, Robert MC. Serum drops for ocular surface disease: national survey of Canadian cornea specialists. *Can J Ophthalmol.* 2018 Jun;53(3):266-271.
61. Schiffman RM, Christianson MD, Jacobsen G, Hirsch JD, Reis BL. Reliability and validity of the Ocular Surface Disease Index. *Arch Ophthalmol.* 2000;118:615–621.
62. Miller KL, Walt JG, Mink DR, Satram-Hoang S, Wilson SE, Perry HD, Asbell PA, Pflugfelder SC. Minimal clinically important difference for the ocular surface disease index. *Arch Ophthalmol.* 2010;128:94-101.
63. Schulze MM, Jones DA, Simpson TL. The development of validated bulbar redness grading scales. *Optom Vis Sci* 2007;84:976-83.
64. Amparo F, Dastjerdi MH, Okanobo A, Ferrari G, Smaga L, Hamrah P, Jurkunas U, Schaumberg DA, Dana R. Topical Interleukin 1 Receptor Antagonist for Treatment of Dry Eye Disease: A Randomized Clinical Trial. *JAMA Ophthalmol.* 2013;18:1-9.
65. Lancaster GA, Dodd S, Williamson PR. Design and analysis of pilot studies: recommendations for good practice. *J Eval Clin Pract* 2004;10:307–12.
66. Sim J, Lewis M. The size of a pilot study for a clinical trial should be calculated in relation to considerations of precision and efficiency. *J Clin Epidemiol* 2012;65:301–8.
67. Julious SA. Sample size of 12 per group rule of thumb for a pilot study. *Pharm Stat*2005;4:287–91.