Pilot Study to Examine Efficacy and Cytokine Levels after Meibomian Gland Expression (MGX) with and without Intense Pulsed Light Treatment (IPL)

NCT# NCT03194698

4/28/2017

Pilot Study to Examine Efficacy and Cytokine Levels after Meibomian Gland Expression (MGX) with and without Intense Pulsed Light Treatment (IPL)

Investigators: Joanne Shen MD, Yael Kusne PhD, William Bourne MD, Michael Fautsch PhD, Dave Patel MD, Paul S Keim PhD, Emily Cope PhD

Location: Mayo Clinic Arizona

Chapter 1: Rationale

1.1 Introduction

Dry eye disease (DED) is a common condition that causes ocular discomfort and reduces visual acuity.[1] The two categories of dry eye disease are evaporative dry eye and aqueous deficient dry eye.[2] Both conditions can involve pathology of the meibomian glands, lacrimal glands, lids, tear film, and surface cells.[2, 3] Meibomian gland dysfunction (MGD) is the leading cause of evaporative dry eye[4] and contributes to aqueous deficient dry eye.[5]

Meibomian glands are modified sebaceous glands located along the upper and lower eyelid margins. Twenty to forty glands are located along each lid[6] and secrete meibum, the lipid component of tears.[7] MGD is defined by the International Workshop in Meibomian Gland Dysfunction[4] as the chronic, diffuse abnormality of the meibomian glands, commonly characterized by terminal duct obstruction and/or qualitative/quantitative changes in the glandular secretion. Patients may experience symptoms of eye irritation and clinically observable ocular surface disease and inflammation due to alteration of the tear film.

MGD is a commonly encountered disease by ophthalmologists. The impact of dry eye on quality-of-life is comparable to the effect of moderate to severe angina or dialysis treatment.[8, 9] The goal of MGD therapy is to provide long term improvement of symptoms for patients by improving the quality of meibum, increasing meibum flow, improving tear film stability, and decreasing inflammation. Commonly used therapies include preservative free drops, omega-3 fatty acid supplementation, topical cyclosporine, serum tears, topical azithromycin, oral doxycycline, moisture chambers, intraductal probing, lid margin exfoliation, automated thermal pulsation, warm compresses, and others. Despite the variety of treatment options available, patients often do not experience complete or long term relief of symptoms. Forced meibomian gland expression was first described in 1921 by Dr. Gifford as an effective method of rehabilitating meibomian glands and improving dry eye symptoms in his patient.[10] The eyelid margins are forcefully compressed to express gland contents. Korb et al described an improvement in lipid layer thickness and symptoms in 10 patients with meibomian gland dysfunction treated with MGX.[11] Forced manual expression is painful for patients, and some of them are unable to tolerate the pain.

1.2 Intense Pulsed Light Therapy

Intense pulsed light (IPL) devices have long been used in the field of dermatology to treat acne rosacea, acne vulgaris, hyperpigmentation, essential telangiectasias, unwanted hair, and photodamaged skin. IPL is a high-intensity light source consisting of visible light in the wavelength range of 515–1200 nm. The light is both polychromatic and incoherent.[10] Most IPL dry eye patients receive this treatment as a last resort after trying several other therapies. They often have severe MGD and few to no expressible glands. Treatments are spaced four to six weeks apart, and patients typically receive one to four treatments with no established limit on the number of quarterly maintenance treatments.

Given that the majority of dry eye syndrome is due to MGD, IPL/MGX treatment would theoretically be successful in improving symptoms in the majority of dry eye patients. For this reason, off-label IPL/MGX treatment was attempted to achieve symptom relief even when no significant ocular rosacea was detected in refractory dry eye syndrome in very symptomatic patients who had exhausted traditional modalities of dry eye treatment.

The specific mechanism of IPL therapy in improving dry eye symptoms is unknown. However, various hypotheses exist based on dermatological studies. It is postulated that oxyhemoglobin in blood vessels located on the surface of the skin absorbs light emitted from the flashlamp. The absorption generates heat that coagulates the red blood cells, leading to thrombosis of the blood vessels.[11-14] Several studies have examined changes in inflammatory regulators with the use of IPL. For instance, when using IPL for aged skin, Huang et al (2011) found an upregulation of MMP-1 and a downregulation of TGF-B1; while El-Domyati et al (2015) found no change in TGFB levels with IPL. In studies using IPL for acne vulgaris skin treatment, Ali et al (2013) found an upregulation of TGF-B1 and Taylor et al (2014) found a downregulation of TNFa expression and no change in IL-10 or IL-8. Nevertheless, more data is needed to elucidate these mechanisms, and to our knowledge, no studies examining cytokine changes with the use of IPL for DED have been published.

There are approximately 40 centers performing IPL nationally; however, specific guidelines on selecting the ideal IPL candidate have not been published. Two peer-reviewed studies have been reported to date on the efficacy of combined IPL/MGX for treating MGD as Dr. Rolando Toyos, the ophthalmologist who introduced IPL to dry eye patients, has described.[11-15] In his three-year retrospective review of 91 patient records, Toyos et al. found a statistically significant improvement in tear film break up time (TBUT, p=0.000). Physician-judged improvement in meibum and lid margins was present in 94% and 98% of patients, respectively. Eighty-seven percent of patients showed improvement in clinical signs and 93% had subjective amelioration of their evaporative DED. Thirteen percent of patients experienced an adverse event. Voros and Gupta conducted a retrospective review of 37 patient records and found a statistically significant decrease in scoring of lid margin edema, facial telangiectasia, lid margin vascularity, and improvement in meibum quality score ($P<0.001$). They also found a significant increase in oil flow score and TBUT ($P<0.001$), and a significant decrease in ocular surface disease index scoring ($P<0.001$).

Several prospective trials have been conducted on the efficacy of IPL (without MGX) for treating MGD. Craig et al. (2015) reported that IPL alone was effective in improving the lipid layer and patient symptoms.[16] In 2016, Jiang [17] enrolled 40 patients to receive IPL on day 1, day 15, day 45 and day 75. When compared to baseline measurements, significant improvements were found in both subjective symptomatic assessments and objective measurements of Meibomian gland secretion quality, TBUT, and conjunctival injection. While this study provided evidence of the benefit of IPL on MGD, its presents some limitations including a lack of control group and no analyses of inflammatory markers, or tear cytokines. In 2016, Gupta et al [18] conducted a cohort study of 100 patients with the diagnosis of MGD and DED who underwent an average of 4 IPL sessions. They reported a significant decrease in lid margin edema, lid margin vascularity, meibum viscosity and OSDI score, in addition to a significant increase in oil flow score and TBUT. Furthermore, in our previously published retrospective chart review of 35 patients with DED who were treated with serial IPL/MGX, we found a significant improvement in dry eye symptoms in 89% of patients and improvement in meibomian gland function in 77% of patients.[19]

1.3 Adverse Events

Previous studies have failed to show significant adverse events with the use of IPL for MGD or DED. Jiang et al's (2016) prospective study reported no change in visual acuity, no change in intra ocular pressure (IOP), no changes to pigmentation and no blistering, swelling, redness or hair loss at the surface.[17] A similar lack of adverse events was found in Gupta et al (2016).[18] Additionally, our 2016 retrospective study found IPL to be well tolerated among participants.[19]

1.4 Summary and Hypothesis

Given the aforementioned data and relative lack of prospective studies regarding the use of MGX/IPL in patients with DED, we propose a prospective, randomized, case-controlled clinical pilot study to examine the efficacy of IPL for both subjective and objective measures. 20 patients with DED will be recruited, and will be randomly assigned to one of two groups: MGX alone, or MGX with IPL, and will undergo treatment every 4-6 weeks for 4 total treatment. Subjectively, participants will be scored using dry eye symptom questionnaires, including the OSDI, prior to treatment and post-treatment. Objective measures at baseline and at the end of the study will include tear cytokine levels, impression cytology for inflammatory markers, meibography, tear osmolarity, and ocular microbiome testing (see Chapter 2).

We hypothesize that use of MGX with IPL will lead to greater improvement in subjective dry eye symptoms, improved objective meibomian gland measures, and an upregulation in tear cytokines such as TGF-B1 and downregulation of inflammatory markers, such as IL-10, and improvement of ocular microbiome load, as compared to participants treated with MGX alone.

Given the lack of adverse effects reported in the literature, we do not anticipate study participants will experience adverse effects with proper performance of the IPL and testing procedures. For IPL subjects, masking using IPL-aid for both eyes will be used to protect the ocular structures. After Fitzpatrick skin type assessment, test application of IPL will be performed at the right preauricular skin and evaluated for erythema or skin change prior to

proceeding with the rest of the IPL application. We will record all events and include them in our analyses.

Chapter 2: Study Overview and Design

2.1 Participant Inclusion Criteria

20 subjects with DED (defined as an OSDI > 33) [20] that has been present for at least 1 year will be recruited. **Participants must have signs of ocular rosacea** including meibomian gland disease and marginal lid telangiectasias with no more than 50% Meibomian gland atrophy (MGA), as assessed at baseline using objective measurement analyses (see Chapter 3). Patients who have a history of LASIK procedures, or any previous refractive surgery will be included in this study. Participants who have no prior history or concurrent treatment for DED within the previous 6 months will be included. (6 months out) Additionally, participants will be age matched by decade and sex controlled between groups. Patients may continue systemic and topical treatments for dry eye (including fish oil, flax seed oil, doxycycline, retina A, topical cyclosporine, acne treatments except Accutane) if started 6 months prior to enrollment and must continue on the same dosing until the end of the study.

2.2 Participant Exclusion Criteria

Several exclusion criteria will be included in order to ensure selection of patients who lack systemic conditions, or whose DED is not expected to change due to severity. Subjects without ocular rosacea will be excluded. Participants will be excluded solely based on the following factors: dry eye symptoms that are not alleviated with topical anesthetic (indicating possible neurotrophic eye pain), presence of systemic conditions including Graft versus Host Disease (GVHD) or Stevens - Johnson syndrome (SJS), and/or presence or history of alkali burns. Additionally, participants who wear contact lenses will be excluded, any participant with evidence of >50% MGA, or any participant with prior history or concurrent treatment for DED in the past 6 months.

2.3 Participant Randomization

Once inclusion criteria have been met, patients will be randomized to one of two groups using a random number generator. 10 patients will be randomized to the control group, in which they will receive MGX every 4-6 weeks; and 10 patients will be randomized to the treatment group, in which they will receive MGX and IPL every 4-6 weeks. Biomarker and microbiome labs and data analyzer will be masked to treatment group.

2.4 Data Collection and Analysis

Appointment 1/Baseline. Data will be collected on the participants first visit (“baseline”) and will include the following: OSDI, visual acuity, slit lamp imaging, lissamine green staining, fluorescein, tear film break up time (TBUT), Schirmers 5 minute with anesthesia osmolarity, meibography, Keratograph5 redness evaluation, Lipiview lipid tear film thickness, , tear cytokine evaluation and impression cytology (for flow cytometry of labeled cells for inflammatory markers), and tear sample for ocular microbiome testing . Participants will receive a random

number identifier, and their data will be entered into an Excel Document ("data file") and RedCap database which will be kept on a secure server at Mayo Clinic Arizona.

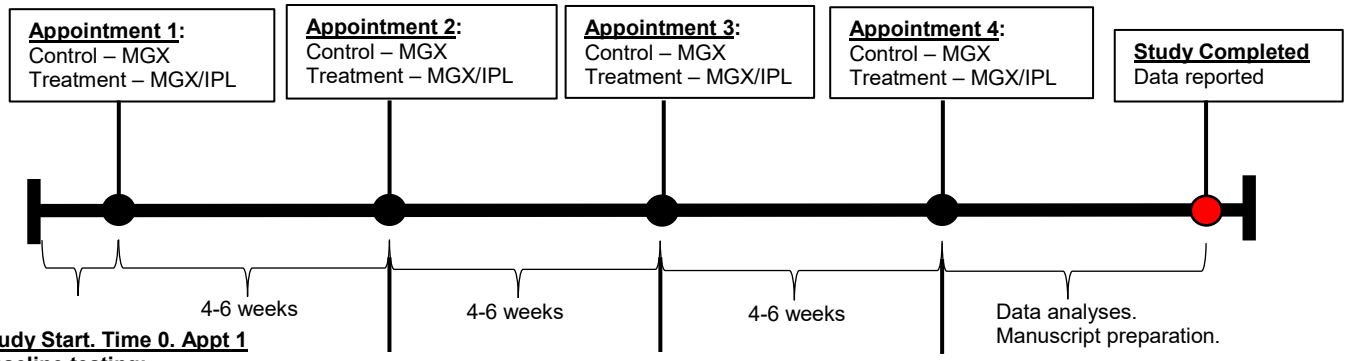
At appointment 1, participants will receive the initial treatment of either MGX or MGX+IPL. At appointment 1, participants will be given Tobramycin-Dexamethasone ophth soln to use BID topically OU for 2 days, and told to avoid UV exposure for 3 weeks.

Appointment 2. 4-6 weeks after appointment 1**,** at appointment 2, participants will be evaluated using OSDI, visual acuity, Icare tonometry, slit lamp imaging, lissamine green staining, fluorescein staining. This data will be entered into the data file. Participants will receive their second treatment of either MGX or MGX+IPL. At appointment 2, participants will be given Tobramycin-Dexamethasone ophth soln BID for 2 days, and told to avoid UV exposure for 3 weeks

Appointment 3. 4-6 weeks after appointment 2**,** at appointment 3, participants will be evaluated using OSDI, visual acuity, Icare tonometry, slit lamp imaging, lissamine green staining, fluorescein staining. This data will be entered into the data file. Participants will receive their second treatment of either MGX or MGX+IPL. At appointment 3, participants will be given Tobramycin-Dexamethasone ophth soln BID for 2 days, and told to avoid UV exposure for 3 weeks

Appointment 4. 4-6 weeks after appointment 3, at appointment 4, participants will be evaluated with the following: OSDI, visual acuity, slit lamp imaging, lissamine green staining, fluorescein, tear film break up time (TBUT), Schirmers 5 minute with anesthesia osmolarity, meibography, Keratograph5 redness evaluation, Lipiview lipid tear film thickness, , tear cytokine evaluation and impression cytology (for flow cytometry of labeled cells for inflammatory markers), and tear sample for ocular microbiome testing. Participants will receive their final treatment of either MGX alone or MGX + IPL. At appointment 4, participants will be given Tobramycin-Dexamethasone ophth soln BID for 2 days, and told to avoid UV exposure for 3 weeks. (Data from Vegunta et al (2016) [19] proved that 3 treatments alone are sufficient to produce a symptomatic MGX/IPL response with improvement of dry eye symptoms and signs. We did not expect that subjects would be compliant to return for a 5^{th} appointment if there was no treatment being performed at that visit.)

Data Analysis. After the completion of 4 treatments, and the collection of data as outlined above, data will be analyzed using statistical methods for changes to the aforementioned tests. The analysis will be conducted by a research trainee who will be blinded to the participant groups. That is, they will receive the identifiers "Group 1" vs "Group 2", without the identification of which group received MGX/IPL vs MGX alone.



Study Start. Time 0. Appt 1
Baseline testing:
- OSDI
- QOL
- VA
- TA
- Lissamine Green
- Fluorescein
- Redness (Keratograph5)
- MMP9
- TBUT
- Schirmers
- Tear Osmolarity
- Meibography
- Lipiview
- Tear cytokine
- Impression Cytology
- Ocular Microbiome

Appt 2 Testing:
- OSDI
- QOL
- VA
- Lissamine Green
- Fluorescein
- TBUT

Appt 3 Testing:
- OSDI
- QOL
- VA
- Lissamine Green
- Fluorescein
- TBUT

Appt 4 Testing:
- OSDI
- QOL
- VA
- TA
- Lissamine Green
- Fluorescein
- Redness (Keratograph5)
- MMP9
- TBUT
- Schirmers
- Tear Osmolarity
- Meibography
- Lipiview
- Tear cytokine
- Impression Cytology
- Ocular Microbiome

Randomization – Control (MGX) vs Treatment (MGX + IPL)

2.5 Design Summary

Power calculation for sample size for biostatistician

Range listed for endpoints

- OSDI (range 2-100)
- QOL
- VA
- Slit lamp
- TA
- Fluorescein (0-15)
- Lissamine Green (0-6)
- Lipiview (20-100)
- Tear Osmolarity (280-320)
- Meibography (0-100)
- Redness (Keratograph5)
- TBUT (0-10)
- Tear cytokines
- Impression Cytology for inflammatory markers
- Ocular microbiome testing

Chapter 3: Experimental Procedures and Cost

3.1 Subjective Parameters

Ocular Surface Disease Index (OSDI). OSDI is the National Eye Institute Visual Functioning Questionnaire and has been proven to be valid and reliable instrument for assessing DED. A copy has been attached in Chapter 4. The range of results is 2-100. We hypothesize a decrease in OSDI levels with MGX/IPL versus MGX alone. Cost for OSDI is estimated at $20 for each test and will be bundled into OPH EST LIMITED EXAM CPT (see 3.3 below). OSDI Survey is included in Chapter 4.1.

QOL Survey.
Cost for QOL is estimated at $20 for each test and will be bundled into OPH EST LIMITED EXAM CPT (see 3.3 below). QOL Survey is included in Chapter 4.2.

3.2 Objective Parameters

Visual Acuity (VA). VA will be recorded in Snellen format for each eye as a safety measure. We hypothesize no changes to VA will be seen between groups. Cost for VA is estimated at $20 for each test and will be bundled into OPH EST LIMITED EXAM CPT (see 3.3 below).

Applanation tonometry (TA). Recorded intraocular pressure (IOP) for each eye as measured by Goldmann applanation tonometry. IOP measurement and recording is a safety measure. We hypothesize no changes to IOP will be seen between groups. Cost for TA is estimated at $20 for each test and will be bundled into OPH EST LIMITED EXAM CPT (see 3.3 below).

Conjunctival Injection Score (Redness). Analyzed using slit lamp images from Keratograph 5. Increased injection has been associated with increased DED symptoms. Score is graded as 0-3, with 0 being no conjunctival injection and 3 being marked injection. We hypothesize a decrease in conjunctival injection score with MGX/IPL versus MGX alone. Cost for conjunctival injection scoring is estimated at $20 for each test and will be bundled into OPH EST LIMITED EXAM CPT (see 3.3 below).

Lissamine Green staining of the interpalpebral conjunctiva. Conjunctival staining is an effective tool to examine epithelial damage. It is easy to perform and has been proven with good reproducibility. We hypothesize an improvement in conjunctival staining with the use of MGX/IPL versus MGX alone. Two areas will be graded from 0-3 in severity. Cost for lissamine green staining is estimated at $20 for each test and will be bundled into OPH EST LIMITED EXAM CPT (see 3.3 below).

Fluorescein Cornea Staining (FCS). Corneal staining is an effective tool to examine epithelial damage. It is easy to perform and has been proven with good reproducibility. We hypothesize an improvement in corneal staining with the use of MGX/IPL versus MGX alone. Five areas

each will be graded from 0-3 in severity. Cost for FCS is estimated at $20 for each test and will be bundled into OPH EST LIMITED EXAM CPT (see 3.3 below).

MMP9. A reliable test used as a marker for inflammation which may provide evidence of ocular surface disease or dry eye with 85% sensitivity and 94% specificity.[21-23] Data has shown abnormal expression of MMP9 expression in DED, which is linked to increased efficacy with the use of anti-inflammatory agents such as cyclosporine or doxycycline.[21, 22] Therefore, this test is a valuable indication of inflammation in DED. Results of the test are reported as positive or negative. We hypothesize that patients treated with MGX/IPL versus MGX alone will show a decrease in MMP9 expression over the course of the trial. Cost for MMP9 testing is estimated at $20 for each test and will be bundled into OPH EST LIMITED EXAM CPT (see 3.3 below).

Tear Break Up Time (TBUT). Considered a mechanism and contributor of DED symptoms [23]. Severe disease criteria cut off is generally considered <3seconds. Split second stopwatch is used with the aid of fluorescein dye. Data has shown TBUT is an DED and is frequently used as a screen for DED.[24] We hypothesize an increase in TBUT with MGX/IPL versus MGX alone. Cost for TBUT is estimated at $20 for each test and will be bundled into OPH EST LIMITED EXAM CPT (see 3.3 below).

Schirmers. Schirmers test is used to evaluate aqueous tear production and therefore is used to aid in possible diagnosis of DED.[23, 25] Results are abnormal if there is <5mm of wetting after 5 minutes. We hypothesize an increase in Schirmer's testing with MGX/IPL versus MGX alone. Cost for Schirmers testing is estimated at $20 for each test and will be bundled into OPH EST LIMITED EXAM CPT (see 3.3 below).

Tear Osmolarity. Studies have shown tear osmolarity to be an important contributor to DED, as it parallels disease severity and responds to treatments.[25, 26] It is, therefore, an accurate and reliable marker of inflammation. Tear osmolarity is evaluated on a continuum, with severe disease being reported as >315 mOsms/L.[26] We expect an improvement (decrease in absolute value) in measures of tear osmolarity with MGX/IPL versus MGX alone. Cost for tear osmolarity is estimated at $20 for each test and will be bundled into OPH EST LIMITED EXAM CPT (see 3.3 below).

Meibography Keratograph. Meibography is a tool to measure the amount of meibomium gland atrophy (MGA). MGA has been reported to be an important cause of dry eye.[27, 28] Images are objectively measured using ImageJ (NIH) based on a previously published report.[29] Results are reported from 0-100% atrophy of total surface area. We hypothesize a significant improvement in MGA with MGX/IPL versus MGX alone. Cost for meibography is estimated at $20 for each test and will be bundled into OPH EST LIMITED EXAM CPT (see 3.3 below).

Lipiview. Lipiview measures lipid tear film thickness by interferometry on Lipiview instrument. Results are reported on a scale from 20-100. We hypothesize a decrease in the absolute

thickness of tear film lipid layer with MGX/IPL versus MGX alone. Cost for lipiview is estimated at $20 for each test and will be bundled into OPH EST LIMITED EXAM CPT (see 3.3 below).

Tear Cytokine. Tears will be collected at baseline and after the third treatment to be analyzed for the following markers: IL-1B, IL-6, IL-8, IL-10, IL-17, IFNg, TNFa, and TGFB1. Tear cytokine analysis of these markers has previously been shown as a marker of ocular surface inflammation, and is upregulated in DED.[30-33] We hypothesize an increase in TGFB1 and a decrease in the aforementioned inflammatory cytokines with MGX/IPL versus MGX alone. Samples will be collected at baseline and frozen at -80*C until analyzed using a pre-made custom plate (Millipore, USA). For 40 samples (20 patients x 2 samples each (1 pre and 1 post treatment), we will require 1 96-well plate at the cost of $1500 + $260 service fees. Additionally, to analyze TGFB1, we will require a separate plate at the cost of $500 + $300 for all samples. Therefore, the final cost for tear cytokine analysis will be $2,560 for all samples for the entirety of the study.

Impression Cytology. Impression cytology is used to sample conjunctival epithelium which has been shown to be affected in DED. [34, 35] The test is minimally invasive, and is well validated within the literature.[34, 35] Using flow cytometry, the expression of 4 markers: HLA-DR (an inflammatory marker), Fas antigen (CD95), Fas ligand, and APO2-7 (apoptotic markers), will be quantified based on previously published reports.[36] Published studies have shown these markers to be upregulated in DED and therefore a biomarker of ocular surface inflammation. [34, 37] Cost is estimated to be $4,000 for all samples for the entirety of the study, which includes antibodies, consumables and service charges.

Microbiology Identification. This test is used to examine the changes in microbiological flora of the eye. Conjunctival swabs will be obtained from patients and sent for RNA sequencing. Previous studies have shown ocular surface bacteria to be related to various ocular surface disease.[38] Additionally, the presence of *Demodex* has been shown to influence meibomian gland dysfunction, cause inflammation, and lead to changes in tear cytokine levels such as IL-17[39, 40]. Through this method, we will be able to assess changes in the ocular microbiome, including alterations in *Demodex*, with MGX/IPL versus MGX alone. Cost is estimated to be $30.07/sample, with a total cost for all 40 samples $2,500. See Section 5 for detailed protocol.

Pharmacy costs: Acuvail costs total $10,000. This is due to the need for 8 vials of the preservative free ampules to be given for OU BID dosing after each treatment session, totaling 640 vials.

3.3 Budget

Please refer to attached Budget for details.

Chapter 4: Supplementary & Supporting Materials

4.1 OSDI

Ocular Surface Disease Index© (OSDI©)[2]

Ask your patients the following 12 questions, and circle the number in the box that best represents each answer. Then, fill in boxes A, B, C, D, and E according to the instructions beside each.

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

Subtotal score for answers 1 to 5 (A)

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	N/A
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

Subtotal score for answers 6 to 9 (B)

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	N/A
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

Subtotal score for answers 10 to 12 (C)

Add subtotals A, B, and C to obtain D
(D = sum of scores for all questions answered) (D)

Total number of questions answered
(do not include questions answered N/A) (E)

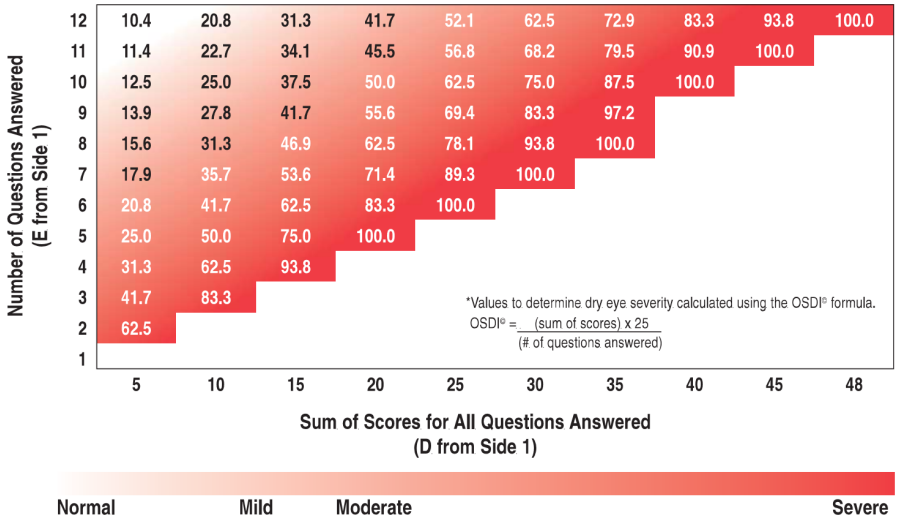
Please turn over the questionnaire to calculate the patient's final OSDI© score.

Evaluating the OSDI© Score[1]

The OSDI© is assessed on a scale of 0 to 100, with higher scores representing greater disability. The index demonstrates sensitivity and specificity in distinguishing between normal subjects and patients with dry eye disease. The OSDI© is a valid and reliable instrument for measuring dry eye disease (normal, mild to moderate, and severe) and effect on vision-related function.

Assessing Your Patient's Dry Eye Disease[1, 2]

Use your answers D and E from side 1 to compare the sum of scores for all questions answered (D) and the number of questions answered (E) with the chart below.* Find where your patient's score would fall. Match the corresponding shade of red to the key below to determine whether your patient's score indicates normal, mild, moderate, or severe dry eye disease.



Patient's Name: _____ Date: _____

How long has the patient experienced dry eye disease? _____

Eye Care Professional's Comments: _____

1. Data on file, Allergan, Inc.
2. 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

4.2 QOL Survey

THE WORLD HEALTH ORGANIZATION QUALITY OF LIFE (WHOQOL) -BREF

The World Health Organization Quality of Life (WHOQOL)-BREF

WHOQOL-BREF

The following questions ask how you feel about your quality of life, health, or other areas of your life. I will read out each question to you, along with the response options. **Please choose the answer that appears most appropriate.** If you are unsure about which response to give to a question, the first response you think of is often the best one.

Please keep in mind your standards, hopes, pleasures and concerns. We ask that you think about your life **in the last four weeks.**

		Very poor	Poor	Neither poor nor good	Good	Very good
1.	How would you rate your quality of life?	1	2	3	4	5

		Very dissatisfied	Dissatisfied	Neither satisfied nor dissatisfied	Satisfied	Very satisfied
2.	How satisfied are you with your health?	1	2	3	4	5

The following questions ask about **how much** you have experienced certain things in the last four weeks.

		Not at all	A little	A moderate amount	Very much	An extreme amount
3.	To what extent do you feel that physical pain prevents you from doing what you need to do?	5	4	3	2	1
4.	How much do you need any medical treatment to function in your daily life?	5	4	3	2	1
5.	How much do you enjoy life?	1	2	3	4	5
6.	To what extent do you feel your life to be meaningful?	1	2	3	4	5

		Not at all	A little	A moderate amount	Very much	Extremely
7.	How well are you able to concentrate?	1	2	3	4	5
8.	How safe do you feel in your daily life?	1	2	3	4	5
9.	How healthy is your physical environment?	1	2	3	4	5

The following questions ask about how completely you experience or were able to do certain things in the last four weeks.

		Not at all	A little	Moderately	Mostly	Completely
10.	Do you have enough energy for everyday life?	1	2	3	4	5
11.	Are you able to accept your bodily appearance?	1	2	3	4	5
12.	Have you enough money to meet your needs?	1	2	3	4	5
13.	How available to you is the information that you need in your day-to-day life?	1	2	3	4	5
14.	To what extent do you have the opportunity for leisure activities?	1	2	3	4	5

		Very poor	Poor	Neither poor nor good	Good	Very good
15.	How well are you able to get around?	1	2	3	4	5

		Very dissatisfied	Dissatisfied	Neither satisfied nor dissatisfied	Satisfied	Very satisfied
16.	How satisfied are you with your sleep?	1	2	3	4	5
17.	How satisfied are you with your ability to perform your daily living activities?	1	2	3	4	5
18.	How satisfied are you with your capacity for work?	1	2	3	4	5
19.	How satisfied are you with yourself?	1	2	3	4	5

20.	How satisfied are you with your personal relationships?	1	2	3	4	5
21.	How satisfied are you with your sex life?	1	2	3	4	5
22.	How satisfied are you with the support you get from your friends?	1	2	3	4	5
23.	How satisfied are you with the conditions of your living place?	1	2	3	4	5
24.	How satisfied are you with your access to health services?	1	2	3	4	5
25.	How satisfied are you with your transport?	1	2	3	4	5

The following question refers to how often you have felt or experienced certain things in the last four weeks.

		Never	Seldom	Quite often	Very often	Always
26.	How often do you have negative feelings such as blue mood, despair, anxiety, depression?	5	4	3	2	1

Do you have any comments about the assessment?

_____

_____

[The following table should be completed after the interview is finished]

		Equations for computing domain scores	Raw score	Transformed scores*	
				4-20	0-100
27.	**Domain 1**	(6-Q3) + (6-Q4) + Q10 + Q15 + Q16 + Q17 + Q18 ☐ + ☐ + ☐ + ☐ + ☐ + ☐ + ☐	a. =	b:	c:
28.	**Domain 2**	Q5 + Q6 + Q7 + Q11 + Q19 + (6-Q26) ☐ + ☐ + ☐ + ☐ + ☐ + ☐	a. =	b:	c:
29.	**Domain 3**	Q20 + Q21 + Q22 ☐ + ☐ + ☐	a. =	b:	c:
30.	**Domain 4**	Q8 + Q9 + Q12 + Q13 + Q14 + Q23 + Q24 + Q25 ☐ + ☐ + ☐ + ☐ + ☐ + ☐ + ☐ + ☐	a. =	b:	c:

* See Procedures Manual, pages 13-15

5.0 Ocular Microbiome Protocol (NAU Sarah Cope/Paul Keim Lab)

Microbiome samples from meibomian gland expressed material were collected in the office under strict aseptic precautions in the ophthalmology clinic using sterile gloves and instrumentation. Material was collected and transferred to sterile swab for right eye and left eye samples (COPAN LQ Stuart Transport Swab, COPAN Italia S.p.A, Brescia, Italy). Any contaminated swabs were discarded and repeat sampling performed. After collection, the swab tips were cut with sterilized scissors and placed into Eppendorf tubes and placed in dry ice container. The samples were immediately sent for freezing frozen in a −90°C bath of Novec engineered fluid (3M HFE-7000) cooled in a HistoChill freezing bath (SP Scientific HC80A0). The time from the start of harvest to freezing was approximately 10 -15 minutes. No saline, embedding medium or preservative was added. Unique identification numbers were assigned to each individual container with bar code labels. Biospecimens were stored in ThermoFisher Ultra Low Temp upright freezers at -80°C until shipped to Keim/Cope lab at NAU on dry ice and then stored at -80°C until ready for analysis.

DNA Extraction

Total genomic DNA was extracted using the QIAamp DNA Mini Kit (Qiagen; 51304) with some prior modifications. Briefly, swab heads were placed in sterile 2mL microcentrifuge tubes with 400uL of lysis buffer (20mM Tris-HCl [pH 8.0], 2mM EDTA, 1.2% Triton X-100) containing 1mg/mL Lysozyme (Sigma-Aldrich; L7773), 30U/mL Lysostaphin (Sigma-Aldrich; L2898) and 375U/mL Mutanolysin (Sigma-Aldrich; M4782). Specimens were incubated at 37°C for 1 hour and both the lysates and the swabs were transferred to bead-beating tubes containing 373mg of 0.1mm-diameter zirconia/silica beads (Biospec; 11079101z). Bead-beating was performed at 2100 rpm for 1 min using a Digital Vortex Mixer (Fisher Scientific; 0215370) with a vortex adapter (Mo-Bio; 1300-V1-24). The samples were centrifuged at 12,000xg for 5 minutes and the supernatant was transferred to sterile 1.7mL microcentrifuge tubes. Then, 20uL Proteinase K (20mg/mL) and 400uL Buffer AL were added to each sample and incubated at 56°C for 30 minutes. Further DNA purification was performed as described by the manufacturer.

Library Preparation

16S primers that encompass the V3 and V4 region were selected as the region for library preparation[18]. The primer pair, S-D-Bact-0341-b-S-17 and S-D-Bact-0785-a-A-21, corresponds to a region of 16S that on average spans 459 base pairs. The primers were constructed with universal tail (UT) sequences [19]. We modified the protocol from a single index to a dual index approach. Briefly, the common Illumina PCR primer was replaced with a primer containing a second index sequence. Each UT1 and UT2 index was used only once, the advantage of which will be addressed in the discussion. Preparing libraries with universal tails is a 2-step process: target specific amplification and an extension PCR to add the barcodes and Illumina adapter sequences. The target specific primers, in this case 16S primers with universal tails, can be found in Table 1. The target specific amplification in a 25 μl reaction contained 12.5 μl Q5 Hot Start High-Fidelity 2X Master Mix (New England Biolabs Inc.), 1.25 μl of each primer at a stock concentration of 10 μM (final concentration: 500 nM/primer), and 10 μl of DNA with the following PCR conditions: 1. Initial denaturation at 95°C for 3 min; 2. 25 cycles of 95°C for 40 sec, 55°C for 2 min, 72°C for 60 sec; 3. Final extension at 72°C for 7 min. The target specific amplicon was purified with Agencourt AMPure XP beads (Beckman Coulter) according to the manufacturer's protocol at a 1:1 ratio based on volume. The DNA was eluted from the beads in 25 μl of 10mM Tris-HCl + 0.05% Tween 20 solution.

The indexing PCR contained 12.5 μl KAPA HiFi HotStart ReadyMix (KAPA biosystems), 1 μl each of barcoded UT1 and UT2 indexing primers at stock concentrations of 10μM (final: 400 nM/primer) and 10.5 μl of template from target specific PCR at a final volume of 25 μl. The PCR conditions were: 1. Initial denaturation at 98°C for 2 min; 2. 6 cycles of 98°C for 30 sec, 65°C for 20 sec, 72°C for 30 sec; 3. Final extension at 72°C for 5 min. The final product was purified with Agencourt AMPure XP beads as previously described above and the DNA was eluted in 35 μl of Tris-HCl 0.05% Tween 20 solution. The indexed libraries were electrophoresed on a 2% agarose gel at 100V for 1 hour to separate the human mitochondrial 16S (~450 bp) libraries from the bacterial 16S libraries. Gel extraction of the bacterial 16S libraries was performed using the QIAquick Gel Extraction Kit (Qiagen; 28704) following the manufacturer's instructions.

Library Quantification and Sequencing

Individually Indexed libraries were quantified using KAPA Library Quantification Kit—Illumina/ABI Prism (KAPA biosystems) qPCR. The samples were pooled at equimolar concentrations to enable efficient multiplexing during the sequencing. The final library pool was quantified using the same method and was used for the individual libraries. The final library pool was mixed with a phiX control library a phiX control library (Illumina; FC-110-3001) at 25% of the total library material, as per Illumina's instructions for amplicon sequencing, and was loaded onto the Illumina MiSeq at 14pM. PhiX provides the base diversity that the Illumina instrument needs for proper calculations. The library pool was sequenced with 300 bp paired end reads using version 3 chemistry (Illumina; MS-102-3003). Custom sequencing primers for the dual indexed UT libraries were added to the appropriate wells in the MiSeq cartridge at a concentration of 0.5 μM.

Sequence Processing

Paired end reads for each sample were assembled using SeqPrep. The following changes were made to the default SeqPrep settings: "-L 400", "-n 1", "-A CAAGCAGAAGACGGCATACGAGAT", and "-B AATGATACGGCGACCACCGAGATCTACAC". The merged reads were clustered into operational taxonomic units (OTUs) at 99% by identity against the greengenes database with QIIME (1.9.1) using a previously described protocol (default parameters and tools unless otherwise noted)[21]. Reads that failed to hit the reference sequence collection were retained and clustered *de novo*. Sequences were aligned using PyNAST and taxonomy was assigned using uclust in the QIIME environment.

Sequence and Statistical Analysis

To determine the baseline variability of the bacterial microbiota within an individual, the V3-V4 region of the 16S rRNA gene was sequenced on the Illumina MiSeq. All analyses were performed on an operational taxonomic unit (OTU; number of clusters of similar sequences) table rarefied to 5500 sequences/sample. Alpha-diversity, which is used to assess diversity, evenness, and richness in a community in a single sample, was measured to ascertain differences in the microbiota between IM and MM across the spectrum of CRS disease. Alpha diversity indices studied were: Faith's phylogenetic diversity (measure of biodiversity incorporating phylogenetic difference between species) and Shannon diversity (richness and evenness; "evenness" is a measure of relative abundance of different species that make up the richness in that area)]. A permutational t-test (999 Monte Carlo permutations) was used to determine changes in alpha-diversity (diversity within a sample). Alpha diversity values were projected onto an image of the sinonasal cavity on the MM middle or IM using SitePainter[22].

Beta diversity (comparison of samples to each other to measure the distance or dissimilarity between each sample pair) was performed using UniFrac distance matrices generated in QIIME 1.9.0. Average weighted and unweighted UniFrac values were calculated between subjects. Principal Coordinates Analysis (PCoA) plots were used for visualization of the data present in the beta diversity distance matrix using Emperor. Permutational analysis of variance (PERMANOVA) using the adonis function in the R Vegan package was used to determine significance in distance matrices across samples by metadata categories. Procrustes analysis was performed on the first three dimensions of a PCoA generated using a weighted UniFrac distance matrix and a Monte Carlo simulation was performed with 10000 permutations to determine significance. Procrustes sum of squares (m^2) and correlation [$r = \sqrt{(1 - m2)}$] are reported. To confirm the Procrustes findings, a two-sided Mantel test with 10000 permutations was performed on weighted UniFrac distances matrices generated independently for each sample within a pair.

6.0 Intense Pulsed Light – Meibomian Gland Expression (IPL-MGX) treatment protocol

Subjects undergo Fitzpatrick skin typing to classify their skin response to ultraviolet exposure by degree of burning and tanning. Fitzpatrick skin types I, II, III, and IV are included as recommended by the manufacturer, and V and VI were excluded. Quadra Q4 IPL Machine (**DermaMed Solutions, LLC,** Lenni, Pennsylvania) is used for all subjects. Subjects will have ocular rosacea and will not have active lesions, skin cancer, or specific skin pathology that would exclude treatment with IPL.

Subjects will receive four IPL-MGX treatments, each spaced four to six weeks apart. IPL machine will be set to appropriate dry eye settings—either 1D, 2D, or 4A. At each treatment, the eyelids are bilaterally closed and sealed shut with IPL-Aid disposable eye shields (Honeywell Safety Products, Smithfield, Rhode Island). After generous application of ultrasonic gel to the treated skin, subjects receive approximately 30-40 pulses (with slight overlapping applications) from the right preauricular area, across the cheeks and nose to the left preauricular area, treating up to the inferior boundary of the eye shields. Each treatment is followed by MGX with Hardten expression forceps to empty meibum from bilateral upper and lower eyelids. Expressed material is sent for ocular microbiome testing at NAU. Subjects use tobramycin/dexamethasone drops twice a day for two days following IPL-MGX treatment.

7.0 MGX only protocol

Subjects undergo Fitzpatrick skin typing to classify their skin response to ultraviolet exposure by degree of burning and tanning. Fitzpatrick skin types I, II, III, and IV are included. Subjects will have ocular rosacea and will not have active lesions, skin cancer, or specific skin pathology that would exclude treatment with IPL (to ensure case controls for test group).

Subjects will receive four MGX treatments, each spaced four to six weeks apart. Each treatment consists of MGX with Hardten expression forceps to empty meibum from bilateral upper and lower eyelids. Expressed material is sent for ocular microbiome testing at NAU. Subjects use tobramycin/dexamethasone drops twice a day for two days following MGX treatment.

References

1. McGinnigle, S., S.A. Naroo, and F. Eperjesi, *Evaluation of dry eye.* Surv Ophthalmol, 2012. **57**(4): p. 293-316.
2. *The definition and classification of dry eye disease: report of the Definition and Classification Subcommittee of the International Dry Eye Workshop.* Ocular Surface, 2007. **5**: p. 75-92.
3. Labbe, A., F. Brignole-Baudouin, and C. Baudouin, *[Ocular surface investigations in dry eye].* J Fr Ophtalmol, 2007. **30**(1): p. 76-97.
4. Nelson, J.D., et al., *The international workshop on meibomian gland dysfunction: report of the definition and classification subcommittee.* Invest Ophthalmol Vis Sci, 2011. **52**(4): p. 1930-7.
5. Nichols, K.K., et al., *The international workshop on meibomian gland dysfunction: executive summary.* Invest Ophthalmol Vis Sci, 2011. **52**(4): p. 1922-9.
6. Bron, A.J., L. Benjamin, and G.R. Snibson, *Meibomian gland disease. Classification and grading of lid changes.* Eye (Lond), 1991. **5 (Pt 4)**: p. 395-411.
7. Chew, C.K., et al., *An instrument for quantifying meibomian lipid on the lid margin: the Meibometer.* Curr Eye Res, 1993. **12**(3): p. 247-54.
8. Buchholz, P., et al., *Utility assessment to measure the impact of dry eye disease.* Ocul Surf, 2006. **4**(3): p. 155-61.
9. Schiffman, R.M., et al., *Utility assessment among patients with dry eye disease.* Ophthalmology, 2003. **110**(7): p. 1412-9.
10. SR, G., *Meibomian glands in chronic blepharoconjunctivitis.* Am J Ophthalmol 1921. **4**: p. 489-494.
11. Korb, D.R. and J.V. Greiner, *Increase in tear film lipid layer thickness following treatment of meibomian gland dysfunction.* Adv Exp Med Biol, 1994. **350**: p. 293-8.
12. Heymann, W.R., *Intense pulsed light.* J Am Acad Dermatol, 2007. **56**(3): p. 466-7.
13. Papageorgiou, P., et al., *Treatment of rosacea with intense pulsed light: significant improvement and long-lasting results.* Br J Dermatol, 2008. **159**(3): p. 628-32.
14. Mark, K.A., et al., *Objective and quantitative improvement of rosacea-associated erythema after intense pulsed light treatment.* Dermatol Surg, 2003. **29**(6): p. 600-4.
15. Jabs, D.A., et al., *The eye in bone marrow transplantation. III. Conjunctival graft-vs-host disease.* Arch Ophthalmol, 1989. **107**(9): p. 1343-8.
16. Clark, S.M., S.W. Lanigan, and R. Marks, *Laser treatment of erythema and telangiectasia associated with rosacea.* Lasers Med Sci, 2002. **17**(1): p. 26-33.
17. Jiang, X., et al., *Evaluation of the Safety and Effectiveness of Intense Pulsed Light in the Treatment of Meibomian Gland Dysfunction.* J Ophthalmol, 2016. **2016**: p. 1910694.
18. Gupta, P.K., et al., *Outcomes of intense pulsed light therapy for treatment of evaporative dry eye disease.* Can J Ophthalmol, 2016. **51**(4): p. 249-53.
19. Vegunta, S., D. Patel, and J.F. Shen, *Combination Therapy of Intense Pulsed Light Therapy and Meibomian Gland Expression (IPL/MGX) Can Improve Dry Eye Symptoms and Meibomian Gland Function in Patients With Refractory Dry Eye: A Retrospective Analysis.* Cornea, 2016. **35**(3): p. 318-22.
20. Baudouin, C., et al., *Diagnosing the severity of dry eye: a clear and practical algorithm.* Br J Ophthalmol, 2014. **98**(9): p. 1168-76.
21. Chotikavanich, S., et al., *Production and activity of matrix metalloproteinase-9 on the ocular surface increase in dysfunctional tear syndrome.* Invest Ophthalmol Vis Sci, 2009. **50**(7): p. 3203-9.
22. Gurdal, C., et al., *Topical cyclosporine in thyroid orbitopathy-related dry eye: clinical findings, conjunctival epithelial apoptosis, and MMP-9 expression.* Curr Eye Res, 2010. **35**(9): p. 771-7.

23. Dohlman, T.H., J.B. Ciralsky, and E.C. Lai, *Tear film assessments for the diagnosis of dry eye.* Curr Opin Allergy Clin Immunol, 2016. **16**(5): p. 487-91.
24. Shah, S. and H. Jani, *Prevalence and associated factors of dry eye: Our experience in patients above 40 years of age at a Tertiary Care Center.* Oman J Ophthalmol, 2015. **8**(3): p. 151-6.
25. Lemp MA, F.G., *Diagnosis and management of dry eye disease*, in *Duane's Ophthalmology* 2012, Lippincott Williams & Wilkins Philadelphia, PA.
26. Lemp, M.A., et al., *Tear osmolarity in the diagnosis and management of dry eye disease.* Am J Ophthalmol, 2011. **151**(5): p. 792-798 e1.
27. Knop, E., et al., *[Meibomian glands : part III. Dysfunction - argument for a discrete disease entity and as an important cause of dry eye].* Ophthalmologe, 2009. **106**(11): p. 966-79.
28. Finis, D., et al., *Evaluation of Meibomian Gland Dysfunction and Local Distribution of Meibomian Gland Atrophy by Non-contact Infrared Meibography.* Curr Eye Res, 2015. **40**(10): p. 982-9.
29. Pult, H. and B. Riede-Pult, *Comparison of subjective grading and objective assessment in meibography.* Cont Lens Anterior Eye, 2013. **36**(1): p. 22-7.
30. Wei, Y., et al., *Tear cytokine profile as a noninvasive biomarker of inflammation for ocular surface diseases: standard operating procedures.* Invest Ophthalmol Vis Sci, 2013. **54**(13): p. 8327-36.
31. Cocho, L., et al., *Biomarkers in Ocular Chronic Graft Versus Host Disease: Tear Cytokine- and Chemokine-Based Predictive Model.* Invest Ophthalmol Vis Sci, 2016. **57**(2): p. 746-58.
32. Enriquez-de-Salamanca, A., et al., *Tear cytokine and chemokine analysis and clinical correlations in evaporative-type dry eye disease.* Mol Vis, 2010. **16**: p. 862-73.
33. Na, K.S., et al., *Correlations between tear cytokines, chemokines, and soluble receptors and clinical severity of dry eye disease.* Invest Ophthalmol Vis Sci, 2012. **53**(9): p. 5443-50.
34. Epstein, S.P., et al., *HLA-DR expression as a biomarker of inflammation for multicenter clinical trials of ocular surface disease.* Exp Eye Res, 2013. **111**: p. 95-104.
35. Fernandez, K.B., et al., *Modulation of HLA-DR in dry eye patients following 30 days of treatment with a lubricant eyedrop solution.* Clin Ophthalmol, 2015. **9**: p. 1137-45.
36. Brignole, F., et al., *Expression of Fas-Fas ligand antigens and apoptotic marker APO2.7 by the human conjunctival epithelium. Positive correlation with class II HLA DR expression in inflammatory ocular surface disorders.* Exp Eye Res, 1998. **67**(6): p. 687-97.
37. Tsubota, K., et al., *Quantitative analysis of lacrimal gland function, apoptotic figures, Fas and Fas ligand expression of lacrimal glands in dry eye patients.* Exp Eye Res, 2003. **76**(2): p. 233-40.
38. Miller, D. and A. Iovieno, *The role of microbial flora on the ocular surface.* Curr Opin Allergy Clin Immunol, 2009. **9**(5): p. 466-70.
39. Kim, J.T., et al., *Tear cytokines and chemokines in patients with Demodex blepharitis.* Cytokine, 2011. **53**(1): p. 94-9.
40. Kheirkhah, A., et al., *Corneal manifestations of ocular demodex infestation.* Am J Ophthalmol, 2007. **143**(5): p. 743-749.