Eyelid Androgen Treatment in Dry Eye

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Introduction

Dry eye disease (DED), also known as dry eye syndrome, is one of the most common ocular conditions prompting patients to seek eye care. It is prevalent in 5%-50% of the population, depending on the diagnostic criteria and regions of the world investigated.[1] DED has a substantial negative impact on physical, potentially psychological, function and health-related quality of life on those patients.[2] It is also associated with a considerable economic burden and caused an estimated loss of $3.3 billion USD in 2008 in the USA alone.[3]

The management of DED is complicated due to its multifactorial etiology, i.e., whether it is aqueous deficient or evaporative in nature, or a mixture of these two major subtypes.[4] Treatment varies depending on severity, for example, with artificial tears, environmental alteration, and holistic approaches such as omega 3 intake in the early stages, and progressing to drug therapy both topically and systemically as the condition worsens.[5,6]

DED becomes more frequent with age in both women and men, but women are at a higher risk of dry eye than men, which suggests that sex hormones may play a role in this condition.[1] Sex hormones, particularly androgens, have been shown to impact the structure and function of tear apparatus and ocular surface tissues, leading to altered tear, lipid, and mucin production.[7]

Previous studies support the efficacy of androgen treatment in relieving dry eye symptoms and signs related to both aqueous deficient dry eye (ADDE) and evaporative dry eye (EDE). Worda et al.[8] treated one male patient with severe dry eye, probably of the aqueous deficient subtype, although this was not specified, and low serum

testosterone levels, with 3% testosterone cream. The cream was applied topically to the eyelids three times per day (TID) for 3 months which brought tear stability and lipid layer thickness to normal levels.[8] Further, they found no increase in serum testosterone levels from the eyelid application.

Transdermal systemic androgen administration has demonstrated improved signs and symptoms in dry eye patients.[9-11] Two retrospective case series show improvement of dry eye symptoms while receiving systemic androgen replacement in women with low testosterone; one with combined esterified estrogen and methyltestosterone [12] and the other with androgen alone. [9]

Supalaset et al.[10] in a prospective study used conventional systemic transdermal testosterone treatment in postmenopausal women and andropausal men. Following four weeks of treatment, significant improvements in OSDI, tear break-up time, corneal fluorescein staining, and Schirmer test in the testosterone group were found compared to placebo. Further, they demonstrated a systemic increase in testosterone levels in the females (to normal levels) but little change in the andropausal males.

Golebiowski et al.[11] used 1.0% transdermal testosterone cream and 1mg/g estradiol gel applying to the inner thigh daily (QD) to investigate the impact of testosterone and estrogen on dry eye symptoms and signs in postmenopausal women. This randomized, placebo-controlled study recruited 10 subjects in each treatment arm (1.0% testosterone cream, 1mg/g estradiol gel, 1.0% testosterone cream combined with 1mg/g estradiol, and placebo). The results demonstrated increased tear secretion in the testosterone/estradiol combination group for 8 weeks and a strong association between increased serum androgen and improved tear stability in the testosterone group when running the within

group analysis. However, there were no significant changes in total Ocular Comfort Index (OCI) or OSDI symptom scores nor tear function, meibomian gland function, lid morphology, corneal or conjunctival sensitivity in any of the three test arms when compared with the change in the placebo groups after 8 weeks. The results of this study suggested that 1% testosterone may be below the optimal therapeutic concentration, or local application is more effective than systemic transdermal delivery.

Low levels of serum dehydroepiandrosterone (DHEA) have been noted in patients with primary Sjogren syndrome due to the impaired Hypothalamic Pituitary Adrenal (HPA) axis.[13] In a pilot clinical trial of oral DHEA for Sjogren's syndrome, Pillemer et al. found that 200 mg per day DHEA showed no evidence of improvement in dry eye symptoms and objective measures of ocular dryness.[14] Though the examination of serum testosterone level was not conducted in this study, it is possible that the role of systemic serum androgen level is not as important as local sex hormone concentration in the management of dry eye disease. Table 1 summarizes clinical trials of systemic or topical application of androgen treatment in dry eyes. Taken together, these studies show that androgen is a potential treatment for dry eye disease, especially for those with primary androgen deficiency. The question is whether topical androgen treatment either on or in the eye may be effective in both deficient, and non-androgen-deficient individuals.

Table 1
Summary of Clinical Trials of Androgen Treatment in Dry Eye

Author	Subject N (completed)	Subject Type	Route of Administration	Treatment	Duration	Placebo	Effect on Serum Level	Effect on Tear Film
Systemic Administration								
Pillemer 2004 RCT[15]	F=28	Sjogren syndrome	systemic oral DHEA	200mg (purified DHEA, Diosynth Inc, Chicago)	qd for 24 weeks	placebo caps	n/a	no improvement in Schirmer I test and dry eye staining
Nanavaty 2014 [9]	F = 14	androgen deficiency	systemic by thigh	transdermal androgen patch (Intrinsa, P&G UK)	Avg Tx = 13 months	none	144 % increase	Sx improved, TBUT, Schirmer increased
Golebiowski 2017 RCT[11]	F= 40; n = 10 per arm	Menopausal female	systemic by thigh	1% transdermal gel 0.5ml(5mg)	8 weeks	cream or gel; same componen ts sans actives	no change in testo group	Testo group (n = 10): no change in tear rmeasures
Supalaset 2018 RCT [10]	F=34 M=12	androgen deficiency	systemic by adominal skin	50mg Transdermal testosterone (Androgel, Besins Healthcare, Belgium)	4 weeks	100 mg urea cream	Females = 828% increase; Males = 17% increase	vs. placebo: sig. increases in: 1) Sx, 2) TBUT, 3) staining, 4) Schirmer
Ocular Administration								
Worda C 2001[8]	M = 1	KCS	topical cream on eyelid	3% testo cream	three times daily for 3 months	none	24% increase	TBUT, lipid layer improved
Connor et al 2001[16]	F=1 M=9	dry eye	topical eyedrops	1% DHEA drops	4 times daily for 2 weeks	artificial tears	n/a	improved TBUT and Schirmer I
Connor C 2002[17]	F=9 M=11	complains of dry eye	topical eyedrops	1% testo drops VS 1% DHEA drops	4 times daily for 2 weeks	artificial tears	n/a	improved TBUT and schirmer I, DHEA drops work better than artifical tears and testosterone
Connor C 2002 [18]	F=15 M=5	dry eye	topical cream on eyelid	2.5% testos cream	twice daily for 3 weeks	transderm al cream alone	n/a	improved TBUT, Schirmer test, increased contact lens wearing time
Connor C 2003[19]	F=25 M=3	complains of dry eye	topical cream on eyelid	3% testo cream	twice daily for 2 weeks	null transderm al cream	n/a	improved Schirmer test and dry eye symptoms, post menopausal female perceived the greatest relief of symptoms
Connor, C.G. 2004[20]	F=37 M=3	retro symptoms based	topical cream on eyelid	3% testosterone cream	bid for two weeks	None	n/a	TBUT poor predictor, Successful group younger, schirmer test from 7 to 12.72 in successful group(age and lid disease less sensitive)
C.G.,Connor 2005[21]	F=17 M=3	TBUT+Sx	topical cream on eyelid	5% testos cream	twice daily for 3 weeks	None	n/a	increased TBUT(not significant),

								decreased OSDI scores
C.G.,Connor 2006 [22]	F=23		topical cream on eyelid	n/a	twice daily for 3 years	None	n/a	increased TBUT, Schirmer test,increased contact lens wearing time, but IOP not different,
Schiffman R 2006 RCT [23]	179	MGD	topical eyedrops	0.01%, 0.03%, 0.1% drops	6 months	vehicle drop	n/a	0.03% most effective, improved MG secretion quality, no incidence of adverse effect
C.G.,Connor 2007[24]	F=26 M=6	dry eye	topical cream on eyelid	15% progesterone cream	twice daily for 3 weeks	None	n/a	increased TBUT, Schirmer test(not significant), decrease OSDI, but IOP not different
Connor C 2008 [25]	F=21	EDE	topical cream on eyelid	5% testos cream	twice daily for 3 weeks	None	n/a	increased TBUT, Schirmer test(not significant), decrease OSDI
Connor C 2009[26]	F=62	dry eye/age	topical cream on eyelid	5% testos cream	twice daily for 3 weeks	None	n/a	TBUT, Schirmer test, OSDI only significant chagne with 40-60 yo
C.G.,Connor 2010[27]	F=22	reduced CTL time	topical cream on eyelid	5% testos cream	three weeks	None	n/a	increased TBUT, Schirmer test,increased contact lens wearing time
C.G.,Connor 2011[28]	F=24	HRT vs no HRT	topical cream on eyelid	5% testos cream	n/a	None	n/a	Presence of HRT positively influence response
Connor C 2012[29]	F=30	dry eye	topical cream on eyelid	5% testos cream	twice daily for 1 month	none	n/a	increased TBUT from 3.53 to 7.11, no increase in BP

Several studies using topical application of androgen formulation have shown the efficacy of local androgen application to the eyelids on dry eye. Connor et al.[30-32] conducted 3 studies to explore the efficacy of testosterone cream in dry eye patients with or without androgen deficiency. The results showed that the 3% or 5% testosterone cream applied for 2-3 weeks significantly improved the Schirmer's test results and/or the TBUT. The greatest improvement was found in the age group of 40-60 year old women but not in the group over age 60 who are reported to have lower systemic serum androgen levels.[26] The underlying etiology is unknown, but a possible explanation is that androgenic receptor sensitivity in the ocular tissue is increased due to the reduced

androgen level in the 40 to 60 year age group, although serum levels were not measured in this investigation.[26]

In contrast to the systemic endocrine system, human beings have a unique intracrine system to regulate sex hormone production and metabolism locally. Intracrinology describes the local synthesis of androgens and estrogens made locally in each cell of each peripheral tissue from the adrenal precursors dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulfate (DHEA-S).[33] These androgens and estrogens exert their action on the same cells where their synthesis takes place and are released from these target cells only after being inactivated.[34] In humans, DHEA levels in circulation reach maximum levels between age 20 and 30 years, decreasing by 80% by the age of 70 in males and females.[35] The great reduction in adrenal secretion of DHEA and DHEA-S results in a large fall in the formation of androgens and estrogens in peripheral tissue with age.[36] Since the prevalence of DED increases with age, this drop in DHEA, and DHEA-S could be a contributing factor.[7] Thus, intracrinology allows circulation levels of androgen to remain subthreshold while potentially allowing topical application of androgen to play an important role in the management of dry eye disease.

The risk of adverse effects from androgen supplementation is low in individuals with depressed serum androgen levels except for at risk groups such as when breast carcinoma or prostate cancer are suspected. Current commercially available FDA approved transdermal testosterone formulations are indicated for testosterone replacement therapy in males and females with androgen deficiency. Therefore, monitoring serum testosterone concentrations is critical.

The common side effects of excess androgen intake include cardiovascular, musculoskeletal, and psychiatric effects, as well as acne and hair growth.[37] Increases in LDL-cholesterol and reduction in total cholesterol, HDL-cholesterol, and triglycerides have been demonstrated in oral testosterone intake whereas non-oral treatment (via transdermal patch or cream) has no such adverse effects.[37] The meta-analysis reported by Islam et al. [37] indicates testosterone treatment administrated at doses intended to approximate physiological replacement levels is not associated with an increase in serious adverse events in women. Prostate safety is a major concern in men receiving androgen replacement therapy. The current evidence does not support the view that appropriate treatment of hypogonadal elderly men with androgen has a causal relationship with prostate cancer.[38]

Several studies have examined the serum levels of testosterone in relation to studies of dry eye treatment efficacy.[9,11]

Nanavaty et al.[9] applied a transdermal androgen patch in androgen deficient females and found that after three weeks of patch therapy the serum level of testosterone increased approximately 144%, but this brought the serum level into the normal range.[39] Supalaset et al.[10] administered a dose of 50 mg testosterone every other day for four weeks in men and women with decreased serum testosterone levels. They found that serum levels increased approximately 17% in men and 828% in women, but both higher levels were within the normal testosterone range for males and females.[39] Further, 20 percent of androgen-treated subjects reported having oily skin and 4% reported acne.[10]

Golebiowski et al[11] also examined transdermal delivery of testosterone alone, and a testosterone/estradiol combination in postmenopausal women. It is unclear whether the

dose was appropriate for treating low testosterone, but there was no change in serum testosterone level (DHEA-S) in either testosterone-containing arm.[11]

One aspect of local, transdermal application of testosterone to the eyelids is the affected surface area. The upper and lower eyelid surface area is around 8% of one adult handprint (the average surface area of both sides upper and lower eyelid is 14.2 cm^2 (π* 1.5cm*1.5cm*2: the area of upper and lower eyelid is close to a circle with diameter of the length of horizontal eyelid fissure around 3.0 cm. [40]) compared to one handprint 180cm^2 (1% of body surface area BSA 1.8m^2)). The total dosage needed to cover both upper and lower eyelid surfaces is much lower than the systemic application of androgen in the study of Supalaset[10] (98 cm^2; 7cm*14cm: the area the author used to apply androgen cream). The eyelid application is thus a small, and likely safe dosage relative to systemic safety.

There is very little reported data concerning serum testosterone levels following eyelid application. Worda et al.,[8] in one subject who was testosterone deficient, applied 3.0% testosterone cream three times daily and had no change in serum testosterone levels following 2.5 months of drug application. Dr. Charles Connor has conducted several studies[16-21,24,26,31,41] of eyelid testosterone cream application but has not surveyed the serum testosterone changes that might result.

In collaboration with Zhijun Wang, PhD, of the College of Pharmacy at MBKU, we have examined the likely systemic absorption of androgen applied to the upper and lower eyelids BID in the dosage form currently FDA approved for androgen insufficiency (Natesto gel, 11 mg each single application for two eyelids or 22 mg per day total for two

applications to the eyelids per day). This simulation and report is attached as Appendix I (Simulation report, v3, Z Wang, 12-6-21).

The assumption for the simulation was that the amount of gel necessary for one eyelid application was the size of a pea (Dr. Charles Connor, personal communication; email of 11-1-21) which Dr. Zhijun Wang measured to be approximately 0.122 grams. This represents one pump of the Natesto dispenser or 5.5 mg of testosterone delivered.[42] Applied to both eyelids at one application, this represents 11 mg total for a single application.

Beginning on Page 5, the simulation demonstrates for Natesto applied once per day to right and left eyelids, both upper and lower eyelids (11 mg total) that the mean increase in serum levels above baseline of total testosterone is about 0.15 to 0.18 ng/ml, whether for a single application or repeated applications over a 30-day period (Figure 3. A – D). Clark et al.[39] have published the weighted average range values for total testosterone (TT, the parameter that is measured during routine clinical laboratory assay) as 2.54 – 8.90 ng/ml for males and 0.12 – 0.58 ng/ml for females. From the simulation, this indicates that male serum TT increase will be minimal and potentially double the minimum normal level for females, yet still possibly within the normal range for females. It is unlikely that any virilization effects (e.g., facial hair growth, scalp hair loss etc.) will be observed in males and it is unknown whether these same effects will occur in females.

Dr. Connor has monitored patients who were using testosterone cream applied to the eyelids twice a day for at least 3 years and found no elevation in IOP.[41] In another study, Connor observed that short term treatment with 5% transdermal testosterone cream for

dry eye enhances female patient TBUT but doesn't significantly impact the patient's blood pressure.[43]

In our proposed study, we will monitor total serum testosterone levels in all subjects at baseline, and within two hours of the morning eyelid dose at the four-week visit (end of dosing). Until this blood work is accomplished, the effects of 4.5% eyelid testosterone gel applied BID will remain unknown.

Research Rationale

Androgen, especially when topically applied to the eyelids, appears to be effective in management of dry eye disease due to its impact on function of tear apparatus.[7] The results of systemic dermatological application of androgen in the treatment of DED are mixed due to the varying testosterone concentrations and dosage time frames. These studies[9-11] of non-ocular dermatological application of androgen have shown desirable effects and a good safety profile.

In contradistinction, the studies by Worda et al[8] and Connor et al.[16-21,26,31] have examined topical eyelid androgen application with generally favorable effects on tear film parameters and also a good safety profile. However, the eyelid application studies were not consistent in the subject type (e.g., both males and females were not uniformly enrolled), the androgen status and serum levels were not assessed prior to, or following androgen application and the studies were only published as peer-reviewed abstracts and mostly were done in one laboratory. This prospective randomized, controlled double masked study aims to evaluate the efficacy and safety of topical transdermal application to the eyelids of low dosage androgen in a population of patients with evaporative dry eye associated with meibomian gland dystrophy and aqueous tear deficient dry eye.

Methods

Study objective

To evaluate the safety and efficacy of an FDA-approved androgen nasal supplement (Natesto, Acerus Pharmaceuticals Corporation, 4.5% gel concentration) in males and females with dry eye of either aqueous tear deficiency or evaporative subtype.

Clinical hypothesis

- Natesto applied to upper and lower eyelid skin is more effective than placebo as measured by fluorescein tear breakup time (TBUT) and mean scores for meibomian secretion quality at 4 weeks and overall ocular comfort at 8 weeks.
- Natesto has an acceptable safety profile in ocular structures and general systemic system.

Statistical Hypotheses:

H_0: There is no difference in change from baseline to week four for either fluorescein breakup time (TBUT) or meibomian secretion score (0 – 24 scale) between the test and placebo-treated sample.

H_A: There is a difference in the change from baseline to week four for either fluorescein breakup time (TBUT) or meibomian secretion score (0 – 24 scale) between the test and placebo-treated sample

Overall study design

Structure

We propose a randomized controlled trial (RCT) of one month duration dosing BID with an FDA-approved androgen nasal supplement (Natesto, Acerus Pharmaceuticals

Corporation, 4.5% gel concentration) in males and females with dry eye of either aqueous tear deficiency or evaporative subtype.

The test formulation will be marketed Natesto gel, containing 5.5 mg testosterone per pump (17β-Hydroxyandrost-4-en-3-one; inactive ingredients are castor oil, oleoyl polyoxylglycerides and colloidal silicone dioxide; Natesto package insert). The placebo is the exact same formulation without 17β-Hydroxyandrost-4-en-3-one. The drug and placebo will be delivered to and dispensed by Caduceus Medical group in Yorba Linda for MBKU's study use.

Dosage regimen

One dose per eyelid of masked gel, which is one pump from the dispenser, represents 5.5 mg for each eyelid, will be applied on the surface of upper and lower eyelid skin each eye twice per day (separated by 8 hours) for a total daily dose of 22mg.

Duration

2-week screening period; 4-week masked treatment phase, 8-week washout phase.

Overall study duration 14 weeks

Visit schedule (see Table 4 and 5)

- Visit 1: Screening visit (Day -14)
- Visit 2: Baseline (Day 0) : the drug (placebo) is prescribed and dispensed. Instructions are given on how to properly apply the drug. Subjects are instructed to keep the container even if the drug is finished. All containers need to be checked in the following two visits (visit 3 & 4)
- Visit 2a: serum blood draw for baseline total testosterone level, hematocrit, lipid panel, PSA for men only at clinical laboratory

- Visit 3: Two-week visit: check the drug (placebo) to see if supplement needed. Empty containers need to be collected.
- Visit 4: Week 4: the drug (placebo) is discontinued. All dispensed containers need to be collected.
- Visit 4a: serum blood draw for treatment period total testosterone level at clinical laboratory (must be in AM, and must be within two hours of application)
- Visit 5: Week 8: 4-weeks washout phase follow up
- Visit 6: Week 12: 8-week washout phase follow up

Evaluation criteria

Efficacy measures

Primary

- Fluorescein tear breakup time (FTBUT)
- MGD secretion grade (0-24 scale)

Secondary:

1. Schein Questionnaire and Ocular Surface Disease Index (OSDI)
2. Corneal and conjunctival staining as per the Oxford and National Eye Institute (NEI) schemes
3. Tear Meniscus Height (TMH) utilizing Keratography 5M (K5M Oculus, Inc Arlington,WA)
4. Eyelid signs grading of orifice metaplasia, vascularity, plugged orifice, lid margin irregularity, ridging between orifices, and antero-posterior orifice displacement
5. Meiboscore utilizing K5M
6. Schirmer I test without anesthetic

Exploratory:

1. Comparison of National eye Institute (NEI) to Ocular Staining Score (OSS) (DEWS II recommendation) staining scheme for efficiency
2. Repeatability of the above two staining schemes and meibomian gland secretion scores

Pharmacokinetic measures

- The patients will have blood samples (20 ml) drawn for the determination of total serum testosterone. Blood will be drawn at baseline and within two hours of dose application at Week 4 visit.

Safety measures

- Adverse events (treatment related and treatment unrelated)
- Visual acuity (BCVA ETDRS)
- Biomicroscopy
- Ophthalmoscopy exam
- IOP
- Male scalp hair evaluation
- Female hair evaluation (upper lip, chin, cheeks, scalp)

Subjects

Subjects will consist of a convenience sample of patients over 18 years old with signs and symptoms of dry eye disease (DED). Moderate to severe dry eye subjects who have either aqueous tear deficiency or evaporative dry eye related to meibomian gland

dysfunction will be recruited. The diagnosis of DED is made according to the DEWS II diagnostic criteria based on:

1. DE symptomatology: OSDI score ≥ 13 and Modified Schein score ≥ 7.5
2. Plus one of the following:
    a. NIBUT < 10 seconds or fluorescein breakup time ≤ 6.0 seconds or
    b. Osmolarity ≥ 308 mosm/L in either eye or intraocular difference > 8 mosm/L or
    c. Ocular surface staining (SICCA scale) > 5 corneal spots, > 9 conjunctival spots
    d. Conjunctival-corneal epithelial damage (fluorescein, rose bengal, or lissamine green staining score >3 points).
3. Subtype Tests: severity according to the DEWS II Dx report;[4] page 556
    a. Aqueous tear deficiency: non-invasive meniscus height (TMH) ≤ 0.2 mm
    b. Evaporative: only moderate to severe included:
        1. Moderate: 1) orifice plugging, 2) lid margin vascularity, 3) gland secretion grade 8 – 12 (central 8 glands, 0 – 3 scale each), 4) expressibility grade 2
        2. Severe: 1) lid margin dropout or displacement, 2) gland secretion score ≥ 13, 3) expressibility grade of 3

The subjects will be recruited from among students, staff, faculty and patients from Ketchum Health, the clinical facility at Marshall B. Ketchum University. Additionally, the study will be advertised on social media to maximize the recruitment pool, such as Facebook, Instagram, and Twitter. Subjects will be sex-,

and age-matched as they are enrolled since these parameters affect dry eye parameters such as marginal changes and meibomian gland secretion. [44]

Sample size estimation

1. In this present investigation the principal outcome measures, fluorescein TBUT (using 2.0 microliters of 1.0% sodium fluorescein) and the system for meibomian gland secretion (0-24 scale) are recommended by the MGD workshop and DEWS II diagnostic committees.[4] Table 2 shows the sample size estimation with two arm parallel design, comparing the change from baseline in FTBUT in each arm with a two sample t-test continuous data, clinically meaningful change of 2.5 seconds with Standard Deviation (SD) following treatment of 2.5 seconds. The results are as follow:

- 2-Sample t Test
- Testing mean 1 = mean 2 (versus ≠)
- Calculating power for mean 1 = mean 2 + difference
- $\alpha = 0.05$
- Assumed standard deviation = 2.5

Table 2: Sample size estimation

Difference	Sample Size	Target Power	Actual Power
2.0	26	0.8	0.807487
2.5	17	0.8	0.807037
3.0	12	0.8	0.802079
3.5	10	0.8	0.841306

4.0	8	0.8	0.844793

2.

3. With the actual power of 0.807, total of 34 subjects with seventeen (17) subjects per arm will be recruited for this study.

Randomization method

Due to the relatively small sample size ($n < 200$) and the need to control and balance the influence of covariates, such as age and sex, we adopted the method of covariate adaptive randomization.[45] With two covariates of sex (2 levels: male, female) and age (2 levels: <50, >50) between study arms (treatment and control), a total of 4 block combinations is produced. After a participant has passed the screening and day 0 examinations, the first 10 participants will be assigned to one of the treatment groups within each block by simple randomization, according to a randomization scheme (Dr. Andrew Loc Nguyen will generate this). The next new participant is then sequentially assigned to a particular treatment group by considering the specific covariates and previous assignments of participants. For example, the first 10 participants assignment are broken down by covariates as in Table 3. Then the 11th participant, who is male and >50 years old, needs to be assigned to a group (control vs. treatment). Based on the characteristics of the 11th participant, the method adds marginal totals of the corresponding covariate categories for each group and compares the totals. The participant is assigned to the group with the lower covariate total to minimize imbalance. In this example, the appropriate categories are male and > 50 years old, which results in a total of 6 (3 for male and 3 for >50) for the control group and a total of 5 (3 for male and 2 for >50) for the

treatment group. Because the sum of marginal totals is lower in treatment group (5<6), the 11th participant is assigned to the treatment group.

Table 3. Procedure of randomization

Control group		Sex		Marginal total
		Male	Female	
Age	<50	2	0	2
	>50	1	2	3
Marginal total		3	2	5

Treatment group		Sex		Marginal total
		Male	Female	
Age	<50	1	2	3
	>50	2	0	2
Marginal total		3	2	5

Every participant is assigned a number from 001 to 100 and recorded with the specific number during the whole study until de-identified at the end of the study.

Masking

1. The participants will be masked from the assignment of the treatment group.
2. An unmasked research assistant will recruit and schedule subjects and administer the questionnaires and exclude subjects based on the above-mentioned inclusion and exclusion criteria.
3. Another unmasked research assistant will implement the randomization based on the above covariate adaptive randomization method.

4. The principal investigator is masked from tests and placebo formulation during the follow up dry eye evaluations on subjects.
5. All masked information will only be opened at the end of the study or unless an adverse event (AE) requires unmasking to determine whether the AE was due to the treatment medication.

Study Sites

1. Southern California College of Optometry at Main Campus (2575 Yorba Linda Blvd. Fullerton, CA 92831). Main site for dry eye examination

Inclusion & Exclusion Criteria

Inclusion criteria

There are no restrictions for subject inclusion based on ethnicity, race, or occupation. All subjects meet the following criteria:

1. Male or female age over 18
2. Able and willing to follow study instructions.
3. Are able to provide written informed consent
4. Written authorization for use or release of health and research study information will be obtained
5. Are willing to discontinue use of artificial tears and makeup on the study visit days
6. Able and willing to attend the clinical laboratory for morning blood draws to assay serum testosterone in addition PSA levels for men.
7. Patient must have a best corrected visual acuity of 20/40 or better in each eye
8. Patients must have normal lid position and closure

9. Patients' willingness and ability to cooperate with the investigator and follow all instructions
10. A negative urine pregnancy test result for women of childbearing potential
11. Women of childbearing potential (from 18 to 50 years old) must have a history of bilateral tubal ligation or use contraceptives, implants, injectables, transdermal patch or IUD for birth control during the study. Patients taking oral contraceptives must not miss 2 or more doses in a one-month period. If these methods of birth control do not apply, women of childbearing potential must have a monogamous partner who has had a vasectomy at least 3 months before the screening visit. The date of the vasectomy and physician contact information must be documented in the source documents.
12. Subjects must have a meibomian gland secretion score of 8 or greater (0 – 24 scale).
13. Subject s must have at least 50% meibomian glands present in the upper eyelids.

Exclusion criteria

1. Uncontrolled systemic disease or the presence of any significant illness or condition that could, in the judgement of the investigator, jeopardize patient safety or interfere with interpretation of the study results (Sjogren's syndrome and related autoimmune disease are allowed).
2. Known or suspected prostate cancer or male patients with a PSA level > 4ng/mL. If PSA level is > 4 ng/mL, the patient can participate only if the patient receives written approval by a urologist. If a man has had a recent prostate exam, at least 4 weeks should separate the prostate exam and our study screen test. If a man has

benign prostatic hyperplasia (BPH), the participants should be notified the risk of worsening BPH if he wants to participate.

3. Current or recent venous thromboembolism (VTE), including deep vein thrombosis (DVT) and pulmonary embolism (PE). Evaluate subjects who report symptoms of pain, edema, warmth and erythema in the lower extremity or acute shortness of breath and seek medical care.
4. Recent cardiovascular events (e.g., MI, stroke). Subjects who report signs or symptoms of heart attack or stroke should seek medical care.
5. Hepatic disorders (hepatitis, jaundice, hepatic cancer et al.). Subjects should monitor for signs or symptoms of hepatic dysfunction (e.g. jaundice)
6. Subjects with cancer and at risk for hypercalcemia (e.g., lung cancer, kidney cancer, multiple myeloma, lymphoma, leukemia, ovarian cancer. et al.)
7. Subjects taking insulin, warfarin or systemic corticosteroids.
8. Contact lens wear during the six months prior to study start and at any time during the study.
9. Punctal occlusion done within 3 months of the screening visit or during the study
10. Active ocular infection, non-KCS ocular surface inflammation (e.g., episcleritis) or intraocular inflammation (e.g., uveitis)
11. Female patient who is pregnant or nursing or planning a pregnancy during the study.
12. Males or females with a personal history of breast cancer.

13. Patients who are currently on any androgen or anti-androgen treatment or who have discontinued such treatment less than three months prior to the screening visit. Anti-androgen therapy includes medications or supplements that may interfere with testosterone metabolism, such as 5-alpha reductase inhibitors (e.g., finasteride, dutasteride).
14. Patients use of any topical ophthalmic formulations (including over the counter ointments and gels), except the study supplied non-preserved artificial tears (low viscosity Refresh Tears) used as escape medication after the screening visit. Oil and gel-based products are prohibited.
15. Known sensitivity to any components of the study or procedural treatments, including sodium fluorescein
16. History or evidence of herpes keratitis
17. Corneal disorder or abnormalities other than those caused by meibomian gland dysfunction, including those that may affect corneal sensitivity or normal spreading of the tear film (e.g., corneal dystrophy)
18. History of anterior segment surgery or trauma which could affect corneal sensitivities (e.g., cataract surgery, or any surgery involving a limbal or corneal incision within the last 12 months) or patients who have had refractive surgery
19. Patients with CAIS (complete androgen insensitivity syndrome)
20. Ocular surface disease secondary to the destruction of conjunctival goblet cells as with vitamin A deficiency or scarring such as that with cicatricial pemphigoid, limbal stem cell deficiency, graft vs. host disease, alkali burns, Stevens-Johnson syndrome, trachoma, or irradiation

21. Current enrollment in any other clinical trial involving an investigational drug/device or participation in a clinical trial within the last 30 days preceding the screening examination.
22. Patient has a condition or is in a situation which in the investigator's opinion may put the patient at significant risk, may confound the study results, or may interfere significantly with the patient's participation in this study.

Study Procedures

General Procedures

4. All procedures will be conducted on both eyes of the subject. After the completion of data collection and the determination that the data collected on both eyes on all subjects are of equal quality and reliability, the worst eye with the staining score is selected for analysis. If the staining scores for the two eyes are the same, the secretion grade is used as the tie breaker. If both the staining scores and secretion grades are the same, a random eye is selected by flipping a coin. Table 4 is the summary of all related procedures in our study.

Guidelines for Visit Preparation

5. Subjects are required to refrain from using any eye drops or artificial tears for a minimum of 12 hours prior to their visit and dilation drops within 1 week prior to their visit. Subjects will be asked to refrain from any eye rubbing, eye makeup, or facial products (e.g., creams, lotions) near the eyes on the day of the study. Table 5 shows the schedule for all the visits and procedures

Table 4: Procedure summary

Procedure	Notes
Informed Consent / HIPAA	
Medical/CL History	
Visual Acuity	
Questionnaires: OSDI, Schein	**Survey results masked from examining clinician**
Biomicroscopy – lid signs (0 – 5 scale)	**Plugging, notching, ridging, teleangiectasia, metaplasia**
Tear osmolarity	**Tearlab; take higher of R, L values**
NIBUT (Oculus Keratograph)	**3 blinks, hold, average 3 values each eye**
[5 minute rest period to stabilize tear film]	
Tear meniscus height (Oculus Keratograph)	**< 0.2 mm; not specified by DEWS II**
TBUT[a], seconds (fluorescein; nearest 1/10th)	**Mean, median of 3 measurements; alternate eyes; 30 sec. rest periods**
<u>Corneal</u> Staining[a]: Both OSS (0 – 12 system) and NEI (1995) scale (0 – 33) for cornea, conjunctiva	**OSS (SICCA group): 0 – 6 for cornea, 0 – 3 for nasal, temporal conjunctiva) NEI: 0 – 3 score for each of 5 zones on cornea (0 – 15), 6 zones for conj. (0 – 18); Total Scale 0 – 33.**
<u>Conjunctival</u> Staining[b],	**OSS (SICCA group): 0 – 3 for nasal, temporal conjunctiva (0 – 6 total)) NEI: six-zone system. 0 – 3 per zone; 0 – 18 per eye**
Total Staining, for Severity Purposes:	**1. OSS: 0 – 12; if cornea > 5 spots, and conjunctiva > 9 spots = dry eye 2. NEI: 0 – 15 + 0 – 18 = 0 – 33 maximum**
Meibomian Gland Excreta Grading (DEWS II/MGD workshop): lower eyelid (central 8 glands); 0 – 3 scale[c]	**Q-tip or MGE**
Gland Imaging:	**LipiView II or Keratograph 5M; meiboscore in 0.5 scale increments; % gland area present**
Schirmer I sans anesthetic; 5 mins	**Move strip at 2 mins if no wetting[46]**

[a] 2.0 µL of 1% NaFl (micropipette); measure TBUT, then both OSS and NEI within 4 minutes
[b] Lissamine green (strips; wet once, shake off excess; measue within 2 minutes due to elution of dye)
c Bron et al., 1991 and Bron and Foulks, 2003, scale: 0 = clear; 1 = cloudy; 2 -= cloudy with particles, 3 = inspissated/toothpaste

Table 5: Schedule of visits and procedures

Procedure	Screening	0 Week Baseline (Day 0)	2 Weeks	4 Weeks	8 Weeks (4 Weeks washout)	12 Weeks (8 Weeks washout)
Informed Consent / HIPAA	**X**					
Medical/CL History	X	**x**				
Ophthalmoscopy exam		**x**		**x**		**x**
Visual Acuity	X	**x**	**x**	**x**	**x**	**x**
Hair evaluation		**x**		**x**		**x**
Blood Pressure		**x**		**x**		**x**
Blood draw for sex hormone		**x**		**x**		
Questionnaires: OSDI, Schein, MGD-Specific	X	X	X	x	x	X
Biomicroscopy: general, and lid signs (0 – 5 scale)	X	X	X	x	x	X
Tear osmolarity	X	X	X			X
NIBUT (Keratograph)	X	X	X	x	x	X
Tear meniscus height (Keratograph)	X	X	X	x	x	X
TBUT, seconds (fluorescein; nearest 1/10th)	X	X	X	x	x	X

Corneal Staining[a]: Both OSS (0 – 12) and NEI (1995) scale (0 – 33)	OSS and NEI	OSS and NEI	NEI X	NEI x	NEI x	NEI X
Conjunctival Staining	X	X	X	x	x	X
Total Staining, for Severity Purposes:	X	X	X	x	x	X
Meibomian Gland Excreta Grading (0-24)	X	x	X	x	x	X
Gland Imaging:	X			x		X
Schirmer I sans anesthetic; 5 mins	X	**x**	**x**	**x**	**x**	**X**
IOP		**x**		**x**		**x**
Drug or Placebo Application		**Initiate Drug or Placebo Application**		**Halt Drug or Placebo Application**		

Procedure I: Enrollment

- Informed consent and HIPAA documentation and California Research Subject's Bill of Rights. We will discuss the study with subjects and check verbally that they have understood important points, particularly potential risks.
- Comprehensive Ocular and Medical History

- The history questionnaire, which includes questions on contact lens use as well as complete medical and ocular history, will be administered by the examiner and used to further determine whether the subject met the inclusion and exclusion criteria.
- Verify compliance with guidelines for visit preparation
- Best Corrected Visual Acuity (BCVA) (OD/OS)
- BCVA must be 20/25 (0.1 log MAR) or better OD and OS
- LogMAR chart, calibrated at a testing distance of ten feet, and Snellen Equivalent will be recorded.
- If the subject was deemed eligible to participate in the study, they will be asked to participate in Procedure II.

Procedure II: Dry Eye Work-up

- Symptomatology questionnaires (Appendix II Symptoms questionnaires):
- Schein questionnaire;[47]a score of ≥ 7.5 out of 24 indicates dry eye.[48]
- Ocular Surface Disease Index (OSDI);[49,50] a score of ≥13 out of 100 indicates dry eye
    - Subjects will be asked to fill out the questionnaires prior to reporting for the study. The questionnaires will not be scored until after data capture was completed on the subject.
- Tear meniscus height (OD/OS): Measured using Oculus Keratograph 5M: cursor measurement of three lower meniscus heights at approximately 7:00, 6:00 and 5:00
- Tear osmolarity using the Tear Lab method

- Slit Lamp Examination of the health of anterior structures (OD/OS):
    - Upper and lower lids and lashes assessed utilizing white light, diffuse beam (14 mm round), low illumination and 10x magnification
    - Bulbar and palpebral conjunctiva were then assessed using the same settings as those used for lids
    - Corneal health will be assessed utilizing white light parallel parallelepiped beam (~2mm wide, 14 mm tall) at a medium illumination and 16x magnification.
- Eyelid sign grading (OD/OS): Each of the following signs was given a grade of 0 (absent) or 1 (present). A grade of 1 was given if a feature had 2 or more appearances along the lid margin for that eye: [51-53]
    - Orifice metaplasia.
    - Orifice metaplasia: Clear-white filamentous shafts at the gland orifice, evident on direct illumination.
    - Vascularity/ brush marks.
    - Telangiectasia coming from palpebral conjunctiva.
    - Plugged or capped orifices (present if more than one caps on a given eyelid).
    - Ridging between orifices.
    - Eyelid margin irregularity.
    - Notching, ridging at the posterior lid margin border. [these two are equivalent]
    - Anterior or posterior orifice displacement.

- Tear Break Up Time (TBUT) with fluorescein staining (OD/OS): Detailed procedure description below.
- Corneal staining with sodium fluorescein (OD/OS)
- Staining was assessed as per the NEI (Score of 0-15) and OSS (screening and baseline only) schemes for each eye;[54-56] detailed procedure description below.
- Conjunctival Staining with lissamine green (OD/OS):
- Staining was assessed as per the NEI (Score of 0-18) and OSS(Score of 0-12) schemes for each eye; [54-56] detailed procedure description below.
- Secretion Quality (OD/OS):
- Meibomian gland secretion quality will be assessed according to the Bron scale[6,57] utilizing a Meibomian Gland Evaluator (MGE)[58] in order to standardize pressure applied and therefore values measured (gentle rather than a forceful expression);detailed procedure below.
- Schirmer I test, without anesthetic (OD/OS); detailed procedure below.
- Meibography using LipiView II (OD/OS) or K5M keratograpy); detailed procedure below.

Dry eye classification

Data from Procedure II will be analyzed, and each subject will be classified as moderate or severe ADDE or EDE-related to MGD; detailed classification scheme follows.

Detailed Description of Selected Tests

Fluorescein Tear Break-Up Time (TBUT)

6. Liquid sodium fluorescein (NaFl) will be used to visualize the breakup time. The dye will be sourced from Greenpark compounding pharmacy (Houston TX, USA) in 4 ml "droptainers" for study use. An amount of 2.0 µl of 1.0% wt./vol preservative-free NaFl was instilled using sterile pipette tips and a calibrated micropipette. The NaFl dye was stored at 4°C when not in use. Once opened, each bottle of dye was labeled with the date of opening and discarded after 28 days. The slit lamp biomicroscope was utilized to visualize the cornea with high illumination of cobalt blue light, wide beam (~14mm round), and 16x magnification. A yellow #12, 55mm Tiffen filter was held in front of the slit lamp optics to enhance observation of TBUT. A hand-held stopwatch was used to measure TBUT to the nearest $1/10^{th}$ of a second.
7. The examiner has the subjects tilt their head back and look inferiorly while the examiner lifted the right upper eyelid to instill 2.0 µl of 1.0% wt./vol. preservative-free NaFl dye onto the superior bulbar conjunctiva of the right eye, and the process was repeated for the left eye.[55] The subject was then asked to position their head on the patient side of the slit lamp, and close their eyes until the examiner was ready to take a measurement, which took between 45-60 seconds. If there appeared to be inadequate fluorescein in the eye due to rapid drainage or excessive tearing from the subject such that tear break up time could not be measured, the investigator instilled an additional 2.0 µl of 1.0% wt./vol. preservative-free NaFl via pipette and documented on the chart that re-instillation was necessary. The amount of

fluorescein even with re-instillation is so minimal compared to everyday regular clinical practice amounts of fluorescein dye used on the eye that it was unlikely to cause the adverse effects of sequential staining or confound the results.

8. The subject is asked to undertake three natural blinks (i.e., not rapid or "squeeze" blinks) and then to hold the eyelids open while looking at one spot. The first sign of break up (dark spot or change) is recorded to the nearest tenth of a second. Following the first measurement a twenty to thirty second rest period of eye closure is allotted for the patient to re-establish tear stability before measuring the other eye. A minimum of three measurements are taken for each eye with the rest period between each measurement, alternating between eyes. If a measurement is deemed unreliable (i.e., if a subject did not follow instructions, observation is uncertain, or if the tear film contains too much debris) a fourth measurement is taken and the unreliable value is recorded. The mean of the three TBUT measurements, not including the unreliable measurement, is also recorded for each eye.

Corneal Staining

1. The slit lamp used to view the corneal staining is set at maximum illumination on cobalt blue filter, 14 mm round beam, and medium (16x) magnification. Additionally, a yellow #12, 55mm Tiffen filter is used to maximize visibility of the fluorescein staining.
2. Corneal staining is evaluated immediately following TBUT without additional instillation of NaFl solution. Observations are made shortly after TBUT evaluation because the fluorescein diffuses rapidly into the tissue.[55,59,60] If not enough dye is present to evaluate fluorescein staining due to quick lacrimal drainage, study

procedures allow for an additional amount of fluorescein to be instilled prior to grading.

3. National Eye Institute (NEI) systems. The NEI scheme has an integer severity scale of 0 to 3 for each of five corneal sectors for a total corneal scale of 15.[54-56] For NEI schemes, the scales are outlined with illustrated grading panels (Appendix III, Case report form). The scales assess the area, but not depth, of punctate staining, which increase on a log scale, with the highest number being the most staining and most severe. When the examiner graded the level of staining, the grading panels were readily visible and referenced during the evaluation.
4. Corneal staining evaluation is quantified using the Ocular Staining Score (OSS) (only at screening and baseline).[61] Punctate epithelial erosions (PEE) that stain with fluorescein are counted, and scored. If there are no PEE, the score is 0. If 1-5 PEE are seen, the corneal score is 1 (Appendix IV); 6-10 PEE are scored as 2; and >30 PEE are scored as 3. An additional point is added if 1) PEE occurred in the central 4mm diameter portion of the cornea; 2) one or more filaments are seen anywhere on the cornea; or 3) one or more patches of confluent staining, including linear stains, are found anywhere on the cornea. The total fluorescein score for the cornea (the PEE grade plus any extra points for modifiers) is noted in the central square of the SICCA ocular staining score form (Appendix IV). The maximum possible score for each cornea is 6.

Conjunctival Staining

9. A sterile lissamine green strip is wetted with a drop of sterile saline solution. The dye is applied on the superior bulbar conjunctiva on the right eye, followed by the

left eye. The subject is then moved back into the slit lamp headrest, and analysis is performed immediately after the dye was applied. The slit lamp biomicroscope is immediately utilized to visualize the bulbar conjunctiva with moderate high illumination of white light, wide beam (~14mm round), and 16x magnification. The subject is asked to look opposite to the side of the conjunctiva being visualized in order to reveal more of the conjunctiva clearly.

10. Conjunctival staining evaluation is quantified using the OSS and NEI systems. In the OSS, grade 0 is defined as 0-9 dots of lissamine green staining of the interpalpebral bulbar conjunctiva (nasal and temporal conjunctivae graded separately); grade 1 is defined as the presence of 10 to 32 dots; grade 2 as 33 to 100; and grade 3 >100 dots. Because of the difficulty of counting individual dots in a moving eye at the slit lamp, any area of confluent staining >=4 mm^2 is considered to be >100 dots. Nasal and temporal areas of the conjunctiva are graded separately with a maximum score of three for each area or a total maximum score of 6 for each eye. The total OSS for each eye is the summation of the fluorescein score for the cornea and the lissamine green scores for the nasal and temporal bulbar conjunctiva.[61] The NEI scheme has an integer severity scale of 0 to 3 for each of six conjunctival sections (three sections each nasal and temporal sides) for a total corneal scale of 0-18.[54-56] For both schemes, the scales are outlined with illustrated grading panels(Appendix III Case Report form). The scales assess the area, but not depth, of punctate staining, which increase on a log scale, with the highest number being the most staining and most severe. When the examiner grades the level of staining, the grading panels are readily visible and referenced during the evaluation.

Meibum Gland Excreta Grading

1. Procedure: Gland expression is assessed using a slit-lamp biomicroscope at medium (16x) magnification, moderate white light and a wide, 14 mm diffuse beam. A constant pressure device, the Meibomian Gland Evaluator (MGE) developed by Blackie and Korb in 2008[58] is utilized, allowing for standardized and consistent pressure. Prior to gland expression, a Q-tip wetted with non-preserved saline will be used to gently wipe the lid margin to remove any debris.[58] First, the subject is informed that they will feel a tickling sensation on the eyelid margin as it gets wiped with the Q-tip, and the pressure exerted by the MGE is demonstrated on the subject's hand. The subject is instructed to look up and away from the Q-tip or the MGE for the duration of the procedure. To clean the eyelid margin, the investigator gently pulled the skin down by placing the index finger over the edge of the orbital margin in order to prevent any accidental expression of the meibomian glands. Once the eyelid margin is revealed, it is gently wiped with a moistened Q-tip. The investigator held the MGE 1-2 inches from the tip and applied it to the subject's lower eyelid at the base of the lash line to locally push the tarsal plate of the lower eyelid against the globe to express the meibomian glands. As the MGE is applied, it is angled down and the pressure was held for 10 – 15 seconds, watching for oil excretion from the glands to evaluate both the secretion quality and number of glands expressing oil. Expression proceeds targeting approximately 8 glands at the center of lower eyelid. Expression grade and secretion quality are graded individually. The procedure is repeated for each eye.

2. Grading Scales: Grading is based on the average appearance of the center lower eyelid region of a single eye. The Bron scale, initially proposed in 1991,[62] is utilized for Meibomian secretion quality, which uses the color and viscosity of the excreta observed graded on a scale of 0 to 3, as described in Table 6.[6,57]

11.

Table 6: "Bron Scale" Meibomian Gland Expression Grading[6,57]

Grade	Meibum Quality	Meibum Quality Description
Grade 0	Clear	Normal
Grade 1	Cloudy	Diffusely turbid fluid secretions
Grade 2	Granular	Usually turbid fluid secretions, but contains particulate matter. The color of these secretions varies from whitish to yellow.
Grade 3	Inspissated	A semisolid plug or a substance of toothpaste-like consistency; may be extruded as a plug or curled thread. Expression is usually delayed or requires extra pressure. The material contains keratinized epithelial cells.

12.

Schirmer 1 Test

13. The Schirmer test without anesthetic is performed with the subject's eyes gently closed. The subject is told to look upward and blink normally while the strip is placed in the right eye then the left. The Schirmer strips are placed at the junction of the middle and lateral one-third of the lower eyelid. After five minutes the strips are removed, and the amount of wetting is recorded in millimeters. While in the subject's eye, the strip is monitored carefully; if after 2 minutes no wetting is

observed the strip is repositioned for the remainder of the five minutes, and the action is recorded. Due to the discomfort during the procedure, if a subject wets a minimum of 10mm of both strips in less than five minutes, the strips are removed. Care is taken to avoid contaminating the test strip with skin oil from the examiner.[46,59]

Meibography with LipiView II (TearScience, Morrisville, North Carolina, USA)

1. The LipiView II (Tear Science, Morrisville, North Carolina, USA) instrument is used to assess the area of Meibomian gland loss. Images of the subject's eyes are saved on a password protected LipiView instrument consistent with HIPAA. The images are transferred to a flash drive then to a secure research computer for analysis using Image J software for percent Meibomian glands remaining.
2. The subject is seated in front of the instrument with their forehead against the bar. Ambient room lighting is on but slightly dimmed. The lower eyelid is everted using the curved instrument transillumination device to visualize the silhouette of the meibomian glands under the palpebral conjunctiva, and the subject is instructed to look up. The subject's head is turned slightly away from the examiner, until both canthi are equally focused in the frame and the puncta identifiable in the image. Images are then captured. This procedure is repeated for the upper eyelid, using a cotton swab to evert the upper eyelid while instructing the subject to look down. The upper image is taken with reflected IR only, rather than using transillumination lighting, since the instrument does not allow transillumination of the upper eyelids. Both the upper and lower eyelids are imaged since it has been demonstrated that contact lens wear may affect the meibomian glands of both the upper and lower

lids.[63] Additionally, adding the scores of both upper and lower lids proved to have a high AUC sensitivity and specificity in diagnosing MGD.[52]

3. Grading of the meibomian gland loss or dropout used fractions of glands remaining according to the method outlined by Arita *et al*.[52,64] After imaging both the upper and lower eyelids as stated above, the number of partial and complete glands lost was graded according to Table 7 by investigators MA and Jerry Paugh The percent area occupied by the meibomian glands for each eyelid was compared to the percent area affected by partial and total loss of glands for the lower lid only.

14.

Table 7: Meiboscore[52,64]

Grade	**Affected Area**
Grade 0	No loss of meibomian glands
Grade 1	Affected area < 1/3 of total area
Grade 2	Affected area 1/3≤ x ≤ 2/3
Grade 3	Affected area > 2/3

15.

16. Dry eye classification

Subjects are classified as dry eye patients if they met 2 out of the 3 following criteria: TBUT equal to or lower than 6 seconds, dry eye criteria on the NEI staining scale, and dry eye criteria on either meibomian gland expression grade or meibomian gland drop out (Meiboscore). Subjects in Evaporative/MGD group will be classified by severity levels from the international workshop of MGD report of diagnosis subcommittee. [6] Only subjects meeting the criteria of levels 3,4 and 5 will be enrolled. The criteria used to diagnose whether a subject will be enrolled is summarized in

Appendix III: Dry Eye Classification Criteria and Appendix V: Staging the Severity of MGD-Related Ocular Surface Disease

17. General statistical methods and types of analyses

- Comparisons, ANOVA, meibomian secretion scores, meibomian gland dropouts, OSDI
- The primary visit is week 4 and primary efficacy variables are the FTBUT and quality of meibomian secretions
- A two-sided test with p-value less than or equal to 0.05 will be considered as statistically significant.

18. Comparisons between pairs of treatment groups will be done with Cochran-Mantel Haenszel (CMH) tests, stratified by the ocular discomfort strata. Within Strata comparisons will be done with Wilcoxon rank-sum tests for both ordinal and continuous variables. For nominal variables, within strata comparisons will be done with Pearson's chi-square or Fisher's exact tests.

19. Exploratory pharmacokinetics data analysis

- Summary statistics of testosterone, dihydrosterosterone, and 3-alpha-androstanediol glucuronide serum concentrations will be calculated. The relationships between treatment time and gender will be examined.

Confidentiality

The identity of the subjects will be treated as confidential. The results of the study, including any other data, may be published for scientific purposes but will not reveal the name of the subject or include any identifiable references to the subject. Although

measures will be taken to ensure confidentiality, it cannot be guaranteed. Any records or data obtained as a result of subject participation in this study may be inspected by any relevant government agency (e.g., by the US Food and Drug Administration, FDA), by the Marshall B. Ketchum University Institutional Review Board, or by persons conducting this study (providing that such inspectors are legally obligated to protect any identifiable information from public disclosure, except where disclosure is otherwise required by law or a court of competent jurisdiction.) Subject records will be kept confidential in so far as permitted by law. All data from the study will be maintained confidentially on a separate record of the visits where each subject is identified by number identification only. Study records will be kept in a separate file within the locked research storage area at the Paugh laboratory on main campus, and not in the main records area. Any electronic files created that include any patient information or medical data obtained during the study will be kept in password and encryption secured computers of the research staff and Investigators (Drs. Jiang, Paugh, Khankan, Ridder) at the MBKU main campus. These files will not be accessible outside of their respective offices.

Appendix I: see separate word document titled

Appendix II: Symptom Questionnaires

Demographic Data

Subject Name _____ Sex: □ Male □ Female Today's Date: ____ / ____ / ____

Subject's Date of Birth: __ __ / __ __ / __ __ Visit # _____

Time: _____

mm dd yy

Age _____ Subject # _____

Race ☐ White ☐ Black ☐ Asian ☐ Hispanic ☐ Other (specify) _____

Symptomatology Questionnaires

I. Schein Questionnaire, modified by SCCO*

DURING THE PAST WEEK:

1. **Do your eyes ever feel dry?**
   □ Never □ Rarely □ Sometimes □ Often □ All of the time
2. **Do you ever feel a gritty or sandy sensation in your eye?**
   □ Never □ Rarely □ Sometimes □ Often □ All of the time
3. **Do your eyes ever have a burning sensation?**
   □ Never □ Rarely □ Sometimes □ Often □ All of the time
4. **Are your eyes ever red?**
   □ Never □ Rarely □ Sometimes □ Often □ All of the time
5. **Do you notice much crusting on your lashes?**
   □ Never □ Rarely □ Sometimes □ Often □ All of the time
6. **Do your eyes ever get stuck shut in the morning?**

□ Never □ Rarely □ Sometimes □ Often □ All of the time

Total: _____ (score of greater than 5 indicates dry eye)

* a new category of "never" added for Grade 0, and 0-4 scoring added (4 = all the time) from the questions used by Schein OD, Tielsh JM, Munoz MS, Bandeen-Roche K, West S. Relation between the signs and symptoms of dry eye in the elderly. *Ophthalmol*. 1997; 104: 1395-1401.

II. Symptomatology: Ocular Surface Disease Index (from Allergan)

DURING THE PAST WEEK: Have you ever experienced any of the following?

1. **Eyes that are sensitive to light?**

□ All of the time □ Most of the time □ Half of the time □ Some of the time □ None of the time

2. **Eyes that feel gritty?**

□ All of the time □ Most of the time □ Half of the time □ Some of the time □ None of the time

3. **Painful or sore eyes?**

□ All of the time □ Most of the time □ Half of the time □ Some of the time □ None of the time

4. **Blurred vision?**

□ All of the time □ Most of the time □ Half of the time □ Some of the time □ None of the time

5. **Poor vision?**

□ All of the time □ Most of the time □ Half of the time □ Some of the time □ None of the time

Have you had problems with your eyes that limited you in performing any of the following during the last week?

6. **Reading?**

□ All of the time □ Most of the time □ Half of the time □ Some of the time □ None of the time □ N/A

7. **Driving at night?**

□ All of the time □ Most of the time □ Half of the time □ Some of the time □ None of the time □ N/A

8. **Working with a computer or bank machine (ATM)?**

□ All of the time □ Most of the time □ Half of the time □ Some of the time □ None of the time □ N/A

9. **Watching TV?**

□ All of the time □ Most of the time □ Half of the time □ Some of the time □ None of the time □ N/A

Have your eyes felt uncomfortable in any of the following situations during the last week?

10. **Windy conditions?**

□ All of the time □ Most of the time □ Half of the time □ Some of the time □ None of the time □ N/A

11. **Places or areas with low humidity (very dry)?**

□ All of the time □ Most of the time □ Half of the time □ Some of the time □ None of the time □ N/A

12. **Areas that are air conditioned?**

□ All of the time □ Most of the time □ Half of the time □ Some of the time □ None of the time □ N/A

Total: _____ Analysis of Score: _____

*0-4 scoring (0 = none of the time; 1 = some of the time; 2 = half of the time; 3 = most of the time; 4 = all of the time)

(Sum of Scores for All Questions Answered x 100) ÷ (total # of Qs answered X 4.)

Note: any "N/A" questions are not counted in the total # of questions answered

Appendix III: Case report form

I. Demographic Data

Patient Name _____ Sex: □ Male □ Female Today's Date: ____ / ____ / ____

Patient's Date of Birth: __ __ / __ __ / __ __ Age _____

Time: _____

mm dd yy

Race □ White □ Black □ Asian…..□ Hispanic…..□ Other (specify) _____

II. Medical, Eye and Contact Lens History, and Medications

Pertinent Ocular History	**Current Ophthalmic Medications** **(please list medications and reason for use, including artificial tears)**

Contact Lens History	**Ever Worn Lenses?** □ Yes □ No
If lens wear, how long since stopped wearing?	**_____ (for protocol, must have been 12 months of total lens deprivation)**
If Soft (SiHy or Hydrogel)	**Daily Wear?** □ Yes □ No **How Long** (years) _____ **Overnight Wear**? □ Yes □ No **How Long** (years) _____
If Rigid or Gas permeable	**Daily Wear?** □ Yes □ No **How Long** (years) _____ **Overnight Wear**? □ Yes □ No **How Long** (years) _____

Pertinent Medical History	

Benign Prostatic Hyperplasia (BPH)? □ Yes □ No	Prostate Cancer Diagnosis? exclusion criteria□ Yes □ No
Breast Cancer Diagnosis? □ Yes □ No	Allergies? □ Yes □ No List:
History or Symptoms of Venous Thromboembolism? Yes □ No *e.g., **deep vein thrombosis**: pain, edema, redness in lower extremity* *e.g., **pulmonary embolism:** shortness of breath*	History or Symptoms of Polycythemia/erythrocytosis: □ Yes □ No e.g., **erythrocytosis**: HA, dizziness, shortness of breath, nosebleeds e.g., **polycythemia:** fatigue, itching, HA, sweating
Hepatic Changes? (mainly jaundice): □ Yes □ No	Edema: □ Yes □ No
Seborrheic dermatitis? □ Yes □ No	*Acne Rosacea?* □ Yes □ No
Blood thinners? e.g., wayfarin? □ Yes □ No	Recent MI or stroke: exclusion criteria □ Yes □ No
Current Systemic Medications (please list medications and reason for use)	

III. Visual Acuity Snellen / LogMAR (spectacles / unaided; circle one)

O.D. 20 /_____ O.S. 20 /_____

IV. Tear Osmolarity: Measured with TearLab; take greater of R, L value as osmolarity

O.D. _____ O.S. _____ **Visit Osmolarity:** _____ (msOms/L)

V. Interferometry/Blink Analysis (Lipiview, lights dim, warn of flashing lights**)**

OD Avg LLT _____ nm
SD _____

OS Avg LLT _____ nm
SD _____

OD Partial Blinks _____ (20 seconds)

OS Partial Blinks _____ (20 seconds)

VI. Tear Meniscus Height (Oculus Keratograph or reticule eyepiece (at 25X, 1.0 reticule unit = 0.20 mm height))

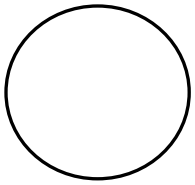
O.D. _____mm

O.S. _____mm

VII. Biomicroscopy, General Examination

OD

Corneal findings:



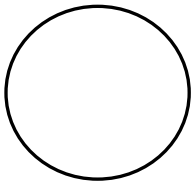
Eyelid findings:

Y N

□ □ orifice metaplasia
□ □ vascularity / brush marks
marks
□ □ plugged orifices
□ □ ridging between orifices
orifices
□ □ anterior/posterior orifice displacement
orifice displacement
(circle one)

OS

Corneal findings:



Eyelid findings:

Y N

□ □ orifice metaplasia
□ □ vascularity / brush
marks
□ □ plugged orifices
□ □ ridging between
orifices
□ □ anterior/posterior
orifice displacement
(circle one)

Total: OD: _____ (0 – 6; score 1 if present)

Total OS: _____ (0 – 6; score 1 if present)

VIII. Non-Invasive and Fluorescein Breakup Time

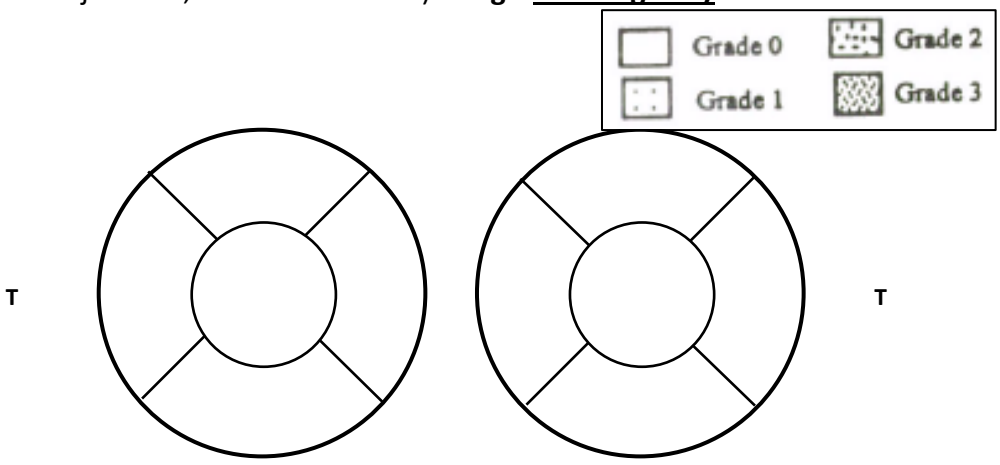
Notes:

1. alternate eyes, 30 seconds rest between measurements to restore tear homeostatic tear stability; ask patient to blink three ***natural*** blinks then hold open, use stopwatch
2. if fluorescein, use Tiffen Yellow 12 filter, wide beam, minimal beam movement

O.D. _____, _____, _____ Average _____ (secs)

O.S. _____, _____, _____ Average _____ (secs)

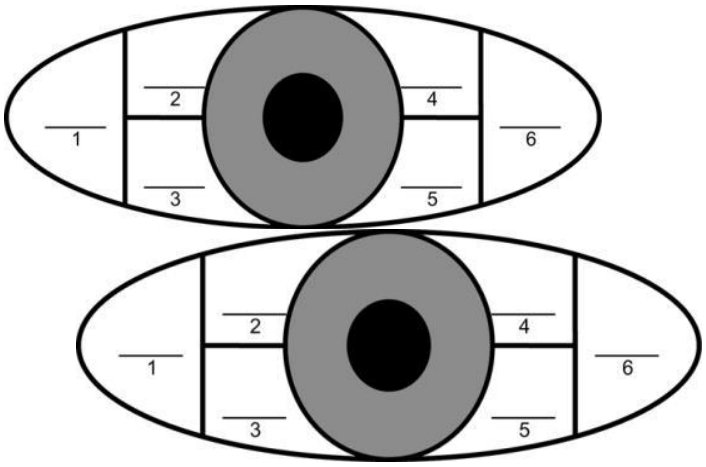
IX. Ocular Surface Staining, NEI (0 – 3 for each of 5 zones for cornea, 0 -3 for six zones (each eye) for conjunctiva, refer to NEI scale) **Integer <u>Grading Only</u>**



Corneal Staining: 0 – 15 for each eye

O.D. _____ O.S. _____

Use lissamine green for conjunctiva (strip; wet with one drop, shake excess)



Conjunctival Staining: 0 – 18 for each eye:

O.D. _____ O.S. _____

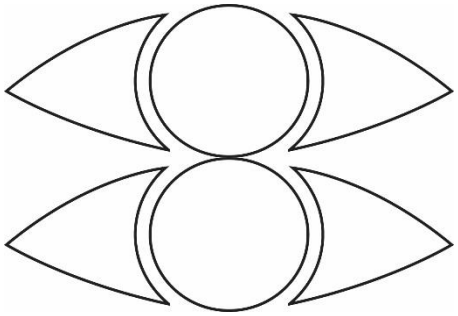
Total NEI Staining: cornea and conjunctiva: 0 – 33:

O.D. _____ O.S. _____

XI. Ocular Surface Staining: sketch the stained areas

Efron: overall stain for cornea, conjunctiva, 0 – 4 scale with 0.5-unit increments

Oxford Scheme: (0 – 5 for each of 3 zones for cornea, nasal, temporal conjunctiva (each eye); refer to separate grading scheme **<u>Integer Grading Only</u>**



Corneal Staining: Fluorescein; 0 - 5

O.D. _____ O.S. _____

Conjunctival Staining: Lissamine green; 0 - 10

O.D. _____ O.S. _____

Total Staining: Efron, 0 – 4 X 3 = 0 – 12; Oxford, 0 – 5 X 3 = 0 - 15

O.D. _____ O.S. _____

X. Meibomian Gland Expression, Lower Eyelid: Q-tip (gentle pressure) or MGE

OD _____ OS _____

Expression, Overall Grade: Bron et al., 1991 scale: 0 = normal, clear; 1 = cloudy; 2 = cloudy with particles; 3 = inspissated, like toothpaste

XI. Meibography Number of images taken:

OD UL _____ OS UL _____

LL _____ LL _____

XII. Meiboscopy, clinical transilluminator **(~8 glands assumed in each sector of eyelid):**

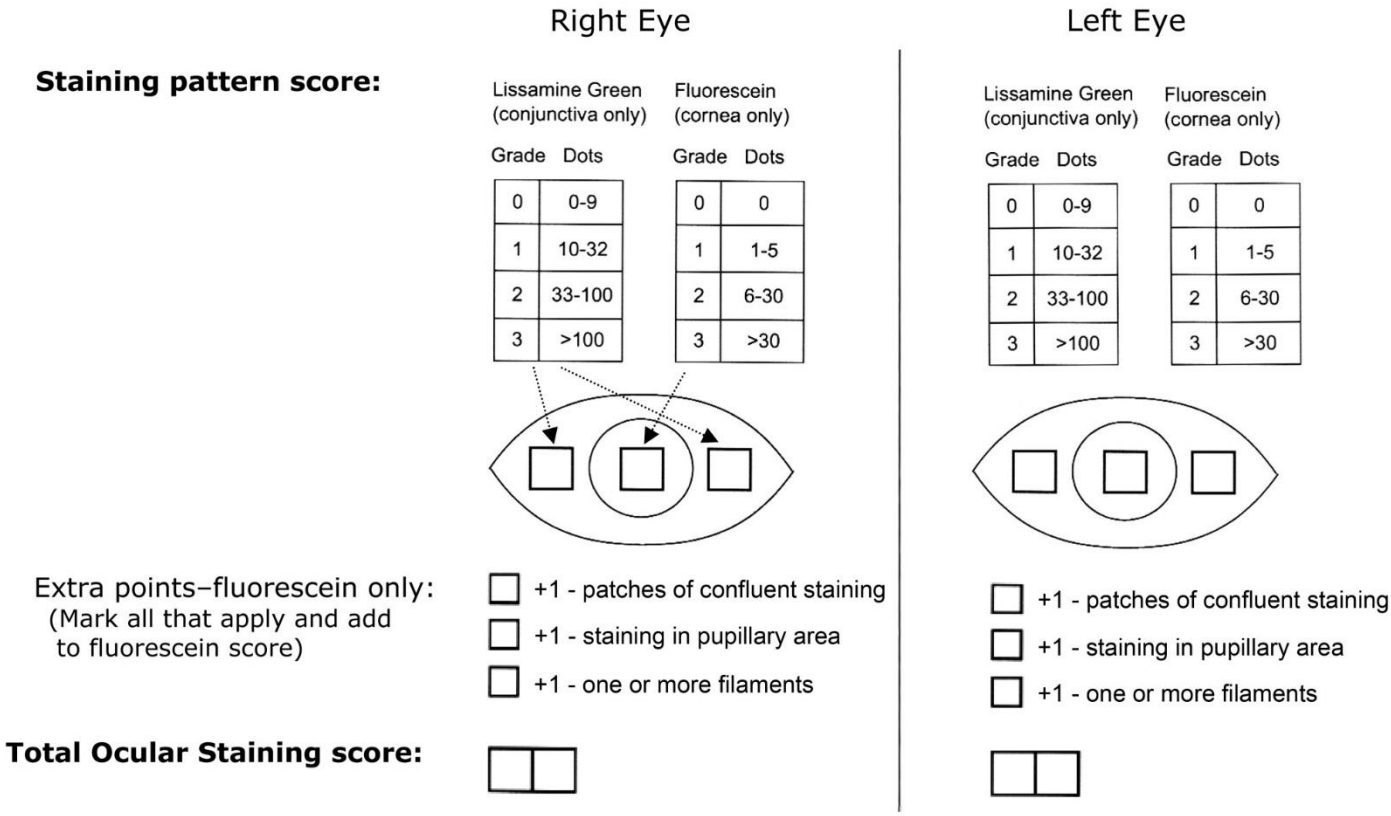
	O.D.	O.S.
Dropout Grade: nasal lower lid	_____	
Dropout Grade: central lower lid	_____	
Dropout Grade: temporal lower lid	_____	
Total Eyelid Dropout Score:	**_____**	**_____**

Dropout: 0 = No Dropout; 1 ≤ 25%; 2 ≤ 50%; 3 ≤ 75%; 4 ≤ 100%; **0.2 grade increments per 5% change**

XV. Schirmer I without anesthetic (amount of wetting in 5 minutes; < 5 mm = ATD)
O.D. _____ (mm) O.S. _____ (mm)

Appendix IV: OSS Staining Form

SICCA Ocular Staining Score



Total ocular staining scores of 0 to 12 per eye assess the range of severity for keratoconjunctivitis sicca

Appendix V Staging the severity of MGD-Related Ocular Surface Disease [6 4]

	Severity					
	Level 0 Normal	Level 1 Subclinical	Level 2 Minimally	Level 3 Mildly	Level 4 Moderately	Level 5 Severely
	No symptoms	Asymptomatic or occasional	Some of the time	Half the time	Most of the time	All the time
OSDI range	0	0-12	0-12	13-22	23-32	33-100
TFBUT s	>=10	<10 to >=7	<7 to >=5	<5 to >=3	<3 to >=1	<1
Tear osmolarity mOsM	Normal <308	Normal <308	Normal <308	<=313 to >308	<= 317 to >314	>317
Grading of MGD Quality(0-24) Expressibility	Quality 0 Expressiblity 0	Quality 0 Expressibility 0	Quality 0-3 Expressibility 0	Quality 4-7 Expressibilty 1	Quality 8-12 Expressibility 2	Quality >13 Expressibility 3
Ocular surface staining NEI Industry scale (0-33)	0	0	0-7	8-14	15-23	24-33
Schirmer Score mm	>=10	>=10	<10 to>=7	<7 to>=5	<5 to >=3	<3

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