A RANDOMIZED, MASKED (EVALUATOR), CONTROLLED, PROSPECTIVE STUDY EVALUATING THE EFFECTIVENESS AND SAFETY OF THE TIXEL MEDICAL DEVICE, VERSUS LIPIFLOW IN THE TREATMENT OF MEIBOMIAN GLAND DYSFUNCTION – STUDY SYNOPSIS

Clinical Investigational Plan (CIP):	**CLN 0858**
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Investigational Product:	**Tixel Fractional System**
Class of Medical Device (EU MDR):	**IIa**
Medical Device Classification (US FDA):	**II**
Sponsor:	**Novoxel Ltd.** **43 Hamelaha St.** **Poleg Ind. Zone, Netanya,** **Israel 4250573**
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1. STUDY SYNOPSIS

Study Name	**A Randomized, Masked (Evaluator), Controlled, Prospective Study evaluating the Effectiveness and Safety of the Tixel® Medical Device, Versus LipiFlow® in the Treatment of Meibomian Gland Dysfunction**
Study Device	Tixel C
Stage of Development	Pivotal study to support an indication for use of treatment of Meibomian Gland Dysfunction
General Design	The study includes 2 stages: stage 1 is the main pivotal protocol, and stage 2 is an extension study for a sub-group population which completed the main protocol, was treated with the Tixel device and meets specific criteria of clinical benefit (specified in the description of stage 2 below).
Stage 1- the main protocol	
Design	• Randomized, open-label study comparing the Tixel device to LipiFlow System. • Up to 110 patients (220 eyes) to be randomized in up to 6 clinical sites in the United States. • Evaluators will be masked as to the randomization assignments. • Both eyes will receive the same randomized assignment and both eyes of each patient will be evaluated at all time points. • Data from both eyes will be using in the statistical analysis. The random-effects model adjusts the standard error (SE) and the confidence interval (CI) for within-person correlation between eyes.
Objective	Evaluate the safety and effectiveness of the Tixel C device in adults with Meibomian Gland Dysfunction (MGD).
Primary Effectiveness Endpoint	Change from baseline to the 4-weeks follow-up exam in Tear Break Up Time (TBUT).
Secondary Effectiveness Endpoints	• Change from baseline in patient symptoms using Ocular Surface Disease Index (OSDI) at 4-weeks and 12-weeks follow-up exam.

	• Change from baseline to 4-weeks and 12-weeks follow-up exam in Meibomian Gland Score (MGS). • Change from baseline to 12-weeks follow-up exam in Tear Break Up Time (TBUT).
Key Safety Endpoint	Comparison of the incidence of Ocular Adverse Events for the two treatment arms.
Secondary Safety Endpoints	• The evaluation of discomfort and pain during treatment. • Changes from baseline following treatment for the test and control devices for the following assessments: ° Ocular Surface Staining. ° Intraocular Pressure. ° Best corrected distance Visual acuity.
Population	Adult patients with symptoms of evaporative dry eye/MGD who do not respond adequately to conservative treatment options such as ocular lubricants.
Sample size	Up to 110 patients (220 eyes), 55 patients per device to be enrolled at up to 6 study sites in the US, will serve as sample size to investigate whether the Tixel C device is non-inferior to the LipiFlow system in terms of change in TBUT from baseline to 4 weeks following the last treatment. Sample size rationale: The sample size calculation is based on the primary effectiveness endpoint. Specifically, the sample size calculation was based on the non-inferiority with a 2.5 seconds' margin of the change from baseline in TBUT score as assessed by a masked rater at 4 weeks follow-up for Tixel versus LipiFlow. The expected standard deviation of the difference in change is 4.5 seconds. The calculation assumes the worst case scenario that the TBUT of both eyes of subjects are completely correlated (r=1). Sample size justification: When the sample size in each group is 44, a two group one-sided 0.05 significance level t-test will have 80% power to reject the null hypothesis that the test and standard are not non-inferior (the difference in means, μ_T-μ_L, is 2.5 s or farther from zero in the same direction) in favor of the alternative

	hypothesis that the means of the two groups are non-inferior, assuming that the expected difference in means is 0 and the common standard deviation is 4.5 s. Assuming a 20% drop-out rate (a relatively high rate is currently assumed due to Covid-19 uncertainty), 55 patients will be enrolled in each group ensuring a sample size of 44 patients per group.
Inclusion Criteria	1. Age 22 years and older of any gender or race. 2. Provision of written informed consent prior to study participation. 3. Willingness and ability to return for all study visits. 4. Reports dry eye symptoms for three months prior to the study. 5. Ocular Surface Disease Index (OSDI) score between 23-79. 6. Tear break-up time (TBUT) <10 seconds in both eyes. 7. Agreement/ability to abstain from dry eye/MGD medications for the time between the treatment visit/s and the final study visit. Ocular lubricants are allowed if no changes are made during the study. 8. Reports having to use artificial tears or lubricants regulatory over the past month to relieve dry eye symptoms. 9. Meibomian gland obstruction in both eyes based on a total Meibomian Gland Secretion Score ≤12 in each eye. 10. At least 15 glands in each lower eyelid should be expressible, with a sterile cotton swab, at the slit lamp.
Exclusion Criteria	1. History of ocular surgery including intraocular, oculo-plastic, corneal or refractive surgery within 6 months. 2. Patients with giant papillary conjunctivitis. 3. Patients with punctal plugs or who have had punctal cautery. 4. Ocular injury or trauma, chemical burns, or limbal stem cell deficiency within 3 months of the baseline examination. 5. Active ocular herpes zoster or simplex of eye or eyelid or a history of these any time. 6. Patients who are aphakic. 7. Cicatricial lid margin disease identified via slit lamp examination, including pemphigoid, symblepharon, etc.

	8. Active ocular infection (e.g., viral, bacterial, mycobacterial, protozoan, or fungal infection of the cornea, conjunctiva, lacrimal gland, lacrimal sac, or eyelids including a hordeolum or stye). 9. Active ocular inflammation or history of chronic, recurrent ocular inflammation within prior 3 months (e.g., retinitis, macular inflammation, choroiditis, uveitis, iritis, scleritis, episcleritis, keratitis). 10. Ocular surface abnormality that may compromise corneal integrity (e.g., prior chemical burn, recurrent corneal erosion, corneal epithelial defect, Grade 3 corneal fluorescein staining, or map dot fingerprint dystrophy). 11. Lid surface abnormalities (e.g., entropion, ectropion, tumor, edema, blepharospasm, lagophthalmos, severe trichiasis, severe ptosis) that may affect lid function in either eye. 12. Anterior blepharitis (staphylococcal, demodex or seborrheic grade 3 or 4). 13. Systemic disease conditions that cause dry eye (e.g., Stevens- Johnson syndrome, vitamin A deficiency, rheumatoid arthritis, Wegener's granulomatosis, sarcoidosis, leukemia, Riley-Day syndrome, systemic lupus erythematosus, Sjogren's syndrome). 14. Use of any of the following medications: a) Systemic medication(s) that is known to cause ocular dryness (e.g., anti-histamines, diuretics, anti-hypertensives, anti-depressants, hormone therapy) whose dose of this medication(s) has not been stable within 30 days prior to enrollment. There must be no anticipated adjustments to the dose of these medications for the duration of the trial. b) Oral tetracyclines or azithromycin within 30 days prior to enrollment; or c) Topical anti-glaucoma medications within 30 days prior to enrollment. d) Any other systemic medication as per to the Investigator's discretion. 15. Women in childbearing age who are pregnant, nursing, or not utilizing adequate birth control measures.

	16. Individuals using isotretinoin (Accutane) within 1 year, cyclosporine-A (Restasis) or lifitegrast ophthalmic solution (Xiidra) within 45 days, or any other dry eye or MGD medications (antibiotics, non-steroidal anti-inflammatory drugs, corticosteroids) for at least 2 weeks; and to maintain abstinence throughout the duration of the study (ocular lubricants are allowed if no changes are made during the study). 17. Individuals wearing contact lenses 1 month prior the study (day 0) and at any point during the study. 18. Current skin cancer, malignant sites and/or advanced premalignant lesions or moles in the treatment area. 19. An impaired immune system condition or use of immunosuppressive medication. 20. Collagen disorders, keloid formation and/or abnormal wound healing. 21. Previous invasive/ablative procedures in the areas to be treated within 3 months prior to initial treatment or plans for such treatment during the course treatment, or before complete healing of such treatments has occurred. 22. Any patient who takes or has taken any oral or topical medications such as but not limited to topical retinoid (e.g., Retin-A), chemical peels, Latisse, Lash Boost which may cause fragile skin or impaired skin healing in the treatment area during the last 3 months and in the entire study period 23. Any patient who has a history of bleeding coagulopathies. 24. Any patient who has tattoos or permanent makeup in the treated area. 25. Any patient who has burned, blistered, irritated or sensitive skin in any of the areas to be treated. 26. Individuals using another ophthalmic investigational device or agent within 30 days of study participation. 27. Any of the following dry eye treatments: a) Office-based dry eye treatment (e.g., IPL, LipiFlow, iLux, TearCare, Tixel, etc.) within 12 months prior to enrollment. b) Meibomian gland expression within 6 months prior to enrollment. c) Blephex or debridement within 3 months prior to enrollment is an exclusion.

	d) Punctal occlusion or punctal plug placement within 30 days prior to enrollment. e) Use of iTear or TrueTear device within the past 2 weeks. (Subjects must refrain from using these devices for the duration of the study.); or f) Any history of meibomian gland probing 28. Use of at-home warm compresses or lid hygiene products while participating in study. 29. IOP higher than 19 mmHg. 30. Use of Botulinum-Toxin in the last 6 months prior to the treatment in the treatment area. 31. Co-existing condition, either ocular or non-ocular that, in the judgement of the investigator could affect the safety or effectiveness of treatment or the compliance of the subject to the protocol.
Schedule of Screening and Baseline Evaluations **For Tixel and LipiFlow Groups**	<u>Screening: (Must be done within 7 days prior to initial treatment (Day 0), but can be also done on the same day as baseline testing and initial treatment)</u> • Informed Consent • Demographics/medical/ocular history • Concomitant systemic and ophthalmic medications • Patient's report of the daily usage (frequency and dose) of eye lubricants • Determination of Fitzpatrick skin type
Pre-Treatment/Baseline	<u>Pre-Treatment/Baseline: (Must be done within 5 days prior to initial treatment but can be also done on the same day. All assessments must be done before randomization and performing either treatment)- the tests must be done in this order and by a masked investigator.</u> • OSDI (administer first, self-assessed) • Refraction • Best Corrected distance visual acuity • Keratometry • Slit Lamp for Anterior Segment Health • Lid Margin Abnormalities

	• Eyelid Margin Assessment (including development of entropion or ectropion, floppy eyelids, lash integrity assessment) • TBUT • Corneal Fluorescein Staining Slit Lamp Evaluation • Conjunctival staining Lissamine green • Meibomian Gland Assessment and Scoring • Meibomian Gland expressibility test- At least 15 glands in each lower eyelid should be expressible, with a sterile cotton swab, at the slit lamp. • Intraocular pressure
Randomization	Randomization: (Performed after determining that the patient is eligible for the study) Patients will be randomized to the Tixel or control LipiFlow group in a 1:1 ratio. Randomization will take place prior to patient treatment. The randomization allocation will be given to the patient directly from the EDC system.
Schedule of Treatments – Tixel Group	
Treatment 1	
Tixel Group	Treatment 1 (Day 0): **Testing must be done in this order:** • Patient's report of the daily usage (frequency and dose) of eye lubricants. If data was collected at screening, only changes will be collected. • Tixel treatment on both eyes per instructions in Testing Methodologies for CLN 0858 study (CLN_0859) and IFU user manual The following assessments will be performed approximately 5 minutes after treatment: • Discomfort and Pain Questionnaires (self-assessed) • Slit Lamp for Anterior Segment Health • Corneal Fluorescein Staining Slit Lamp Evaluation • Conjunctival staining Lissamine green • Intraocular Pressure

	• Assessment for Adverse Events The post Treatment instructions sheet will be given to the patient following initial Tixel treatment.
Schedule of phone call/video call/on-site visit Follow-Up Evaluation	Post treatment 1 checkup call/video call (Day 2 +/- 1 day): The patient will be contacted, first by the study coordinator via phone, 1-3 days post first treatment to check the patient condition. In case of any report of adverse reaction or skin reaction by the patient, the treating physician will contact the patient via video conference or on-site visit. The treating unmasked physician will perform the assessment as per **שגיאה! מקור ההפניה לא נמצא.**.
Treatment 2 & 3 – Tixel Group Only	Treatments 2 and 3: (Must be scheduled 2 weeks (+/-3 days) from the previous treatment). **Testing must be done in this order:** • Concomitant systemic and ophthalmic medications • Patient's report of the daily usage (frequency and dose) of eye lubricants since the previous visit • Slit Lamp for Anterior Segment Health • Lid Margin Abnormalities • Eyelid Margin Assessment • Tixel treatment on both eyes per instructions in Testing Methodologies for CLN 0858 study (CLN_0859) and IFU user manual The following assessments will be performed approximately 5 minutes after treatment: • Discomfort and Pain Questionnaires (self-assessed) • Slit Lamp for Anterior Segment Health • Corneal Fluorescein Staining Slit Lamp Evaluation • Conjunctival staining Lissamine green • Intraocular Pressure • Assessment for Adverse Events
Schedule of Treatments – LipiFlow Group	
LipiFlow Group	Treatment 1 (Day 0): **Testing must be done in this order:**

	• Patient's report of the daily usage (frequency and dose) of eye lubricants. If data was collected at screening, only changes will be collected • LipiFlow treatment on both eyes per instructions in Testing Methodologies for CLN 0858 study (CLN_0859) and IFU. The following assessments will be performed approximately 5 minutes after treatment: • Discomfort and Pain Questionnaires (self-assessed) • Slit Lamp for Anterior Segment Health • Corneal Fluorescein Staining Slit Lamp Evaluation • Conjunctival staining Lissamine green • Intraocular Pressure • Assessment for Adverse Events
Schedule of phone call/video call/on-site visit Follow-Up Evaluation	Post treatment 1 checkup call/video call (Day 2 +/- 1 day): The patient will be contacted, first by the study coordinator via phone, 1-3 days post first treatment to check the patient condition. In case of any report of adverse reaction or skin reaction by the patient, the treating physician will contact the patient via video conference or on-site visit. The treating unmasked physician will perform the assessment as per **שגיאה! מקור ההפניה לא נמצא.**
Follow Up Visits – Applicable for Tixel and LipiFlow Groups	
	Follow-Up Visits: 4-Weeks (+/-7 days) and 12-Weeks (+/-14 days) after the last treatment: the tests must be done in this order and by a masked investigator • OSDI (administer first, self-assessed) • Concomitant systemic and ophthalmic medications • Patient's report of the daily usage (frequency and dose) of eye lubricants since the last treatment • Refraction • Best Corrected distance visual acuity • Keratometry • Slit Lamp for Anterior Segment Health • Lid Margin Abnormalities

	• Eyelid Margin Assessment • TBUT • Corneal Fluorescein Staining Slit Lamp Evaluation • Conjunctival staining Lissamine green • Meibomian Gland Assessment and Scoring (MGS) • Intraocular Pressure • Assessment for Adverse Events
Stage 2- Study Extension	
Study design:	• Single arm observational study for long term follow-up after successful Tixel treatment based on the criteria below. • Only patients who completed their participation in the main study (stage 1) who meet the criteria are eligible to continue to the extension study. • Eligible patients who sign on an additional ICF for participation in stage 2 of the study will undergo a 6-months follow-up visit for effectiveness assessments. Note: Eligible subjects will be able to sign the informed consent form for stage 2 extension study, starting in the 3-months FU visit of the main study and up to the closure of the 6-months FU window. • Once a patient completed the 6-months FU visit he/she completed stage 2 of the study. **Note**: In the main study and the stage 2 extension study, we use both 3-months FU and 12-weeks FU interchangeably.
Extension study endpoint:	Durability of the clinical benefit effect which will be assessed at 6-months FU visit with the same parameters as in the main protocol (OSDI, TBUT and MGSS).
Inclusion criteria:	• Subjects who have completed the main study CLN 0858 (stage 1) in the Tixel arm. • TBUT -change from baseline in 1-month FU or 3-months FU was 2.5 seconds or above at least in one eye in the main study. • Provision of written informed consent for stage 2. • Agreement/ability to abstain from dry eye/MGD medications for the

	time in the extension study. Ocular lubricants are allowed if no changes are made during the study.
Exclusion criteria:	Same as in the main study (stage 1).
Schedule of evaluations:	Screening and 6- months Follow-Up visit: Following completion of the 3-months FU visit in the main study, the investigator will discuss with potentially eligible subjects their continued participation in Stage 2 extension study. Following signing of informed consent for Stage 2 patients will be asked to return to the site 6 months (+/-30 days) after the last treatment for the following assessments (the tests must be done in this order) : All efforts will be done that assessments will be done by the same investigator who did the assessment in the 1-month FU and/or 3-months FU: • Informed consent for stage 2 • Changes in concomitant systemic and ophthalmic medications since the last visit (3-month FU in the main study) • Patient's report of the daily usage (frequency and dose) of eye lubricants (only if changes), since the last visit (3-month FU) in the main study. • OSDI (administer first, self-assessed) • Refraction • Best Corrected distance visual acuity • Keratometry • Slit Lamp for Anterior Segment Health • Lid Margin Abnormalities • Eyelid Margin Assessment • TBUT • Corneal Fluorescein Staining Slit Lamp Evaluation • Conjunctival staining Lissamine green • Meibomian Gland Assessment and Scoring (MGS) • Intraocular Pressure

2. INTRODUCTION

The aim of the current randomized, controlled study is to examine the effect of the Tixel device on the relief of the signs and symptoms of Meibomian Gland Dysfunction (MGD) compared to the FDA-cleared and CE approved device, LipiFlow. The design of this study is a non-inferiority design which is the appropriate design considering the similarity of the mechanisms of the devices and the fact that treatments with LipiFlow commercially available in Europe and the United States for treating MGD. The purpose is to demonstrate that treatment with Tixel is also safe and effective for treating MGD.

The funding for the study is done by the sponsor, Novoxel LTD. Details regarding the funding and the budget are specified in the clinical trial agreement with each site.

2.1.Background

Dry Eye Disease (DED) is often caused by a chronic lack of sufficient lubrication and moisture on the surface of the eye. Consequences of dry eyes range from subtle to constant eye irritation, significant inflammation and even scarring of the front surface of the eye.

Meibomian glands are located in the tarsal plate of the upper and lower eyelids, where they terminate along the interior rim (or margin) of the eyelids. These glands secrete meibum, which is a lipid-rich essential component of a healthy tear film. When sufficient meibum is not present in the tear film, the aqueous layer of the tear film is disrupted and readily evaporates causing irritation, redness, and inflammation of the lid margin and surrounding tissues. Meibomian Gland Dysfunction (MGD) is associated with a failure of these glands to produce adequate quantities of meibum due to atrophy, inflammation, or obstruction and is thought to be the most common cause of evaporative Dry Eye Disease [1; Schaumberg DK 2011].

Initial treatments for the signs and symptoms related to MGD and DED are the use of oil-based artificial tears to supplement the lack of lipid in the tear layer and oral Omega and anti-inflammatory prescriptions to decrease inflammation in the soft tissue in the eyelids. Another common treatment is the use of warm compresses applied over the eyelid for extended periods of time, followed by digital massage of the glands by the patient to try and express oil from the meibomian glands. The results of these treatments are often limited and temporary if not continued on a regular basis.

2.2.Current alternative methods for treating dry eye

Treatments to address this clinical need, aim to provide symptomatic relief, improve quality of life and correct underlying abnormalities of the ocular surface and tear film. The different treatment methods including medicinal (i.e., Lifitegrast), and medical devices such as: IPL, vector thermal pulsation (LipiFlow and i-Lux) and others[1] ; Combination therapy, consisting of dry eye treatment (artificial tears, hyaluronic acid eye drops, Diquafosol ophthalmic solution) and lipid treatment, is effective in the improvement of both subjective and clinical symptoms. In case of microbial infection, MGD (Propionibacterium acnes [P. acnes] and Staphylococcus), antibiotic eye drops are used. Another common treatment is the use of a steroid antimicrobial ophthalmic solution. Treatment with Medical devices utilizing applied heat, such as hot compresses and mechanical pressure to achieve effects, such as:

• Triggering the onset of meibum liquefaction, thus assisting in secretion through the gland orifices.

• Melting of the oil blocking Meibomian glands.

Such devices include LipiFlow (Johnson & Johnson, New Brunswick, NJ), MeiboPatch (VISU pharma, Amsterdam, the Netherlands), Intense pulsed light (IPL) devices, etc. All of them treat DED and MGD. Several technologies have been studied by dermatologists and ophthalmologist regarding the ability of energy-based dermal devices to alleviate DED. Among them are IPL devices[2] . According to Wang et al[3], the complex multifactorial mechanisms of action of IPL therapy for MGD treatment is not fully understood. Both researchers have presented several possible IPL characteristics that may assist. It was proposed that delivery of thermal energy may assist in liquefaction of inspissated meibum and relieve ductal obstruction of the meibomian glands. Alternatively, antimicrobial effects of IPL therapy may dampen inflammatory triggers on the ocular surface. It was also suggested that the heat affects Demodex which influences DED.

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[1] E. C. O'Neil, M. Henderson, M. Massaro-Giordano, V.Y. Bunya, Advances in dry eye disease treatment, Curr Opin Ophthalmol 2019, 30:166–178, DOI:10.1097/ICU.0000000000000569

[2] R. Aritaa, S. Fukuokab, N. Morishigec, Therapeutic efficacy of intense pulsed light in patients with refractory meibomian gland dysfunction, The Ocular Surface Volume 17, Issue 1, January 2019, Pages 104-110, https://doi.org/10.1016/j.jtos.2018.11.004

[3] M. T. M. Wang, U. Khatoon, Jennifer P. Craig, The use of intense pulsed light therapy in the treatment of refractory meibomian gland dysfunction, EXPERT REVIEW OF OPHTHALMOLOGY 2020, VOL. 15, NO. 4, 197–200, https://doi.org/10.1080/17469899.2020.1752666

2.3.Study Rationale

Thermal pulsation has been used in clinical settings to provide heat and mechanical pressure to the eyelids to unblock plugged meibomian glands and reduce symptoms of MGD. Currently there are three FDA-cleared thermal pulsation devices; LipiFlow by Johnson and Johnson (K093937), iLux by Tear Film Innovations, Inc. (K172645), and TearCare by Sight Sciences (K213045). These systems apply heat (approximately between 40° to 45°C) on the eyelids (Lipiflow on the eyelid conjunctiva) and apply gentle pressure on the lid surfaces. Warming the eyelid tissue softens and/or melts the meibum, which is known to facilitate gland expression using pressure.

The Tixel provides another thermal-based solution which has shorter exposure time, with lower thermal energy transfer, lower pressure, and no radiation emission whatsoever. Due to these suggested advantages Novoxel would like to further explore the performance of the device.

Another research motivation has been discovered during research of treatment of periorbital wrinkles: The Tixel device has been used on the skin area around the eyes to reduce periorbital wrinkles. It has been reported by more than a 100 patients treated with the Tixel in some investigator-initiated studies that after treatment their eyes feel less dry. Further clinical information concerning these cases is depicted in the following section.

2.4.Primary Efficacy Endpoint Rationale

The primary objective of the study is to determine the effectiveness of the therapy in improving TBUT in eyes with DED due to MGD. This objective was chosen because a reduced TBUT is one of the most common signs of DED due to MGD. TBUT is considered abnormal when shorter than 10 seconds.

In this study we wish to compare the Tixel device to a marketed device, the LipiFlow system. In the comparative study of iLux vs LipiFlow the chosen NI margin was 2.5 seconds[4]. Since a criterion for clinically relevant improvement in TBUT has not been defined, clinical relevance was based on the labeling of the DET test strip which defines the difference between dry and normal tear stability as 5 seconds. Thus, a change of 2.5 seconds was defined as a clinically relevant change since it represents 50% of that defined in the DET strip labeling.

[4] Tauber J, Owen J, Bloomenstein M, Hovanesian J, Bullimore MA. Comparison of the iLUX and the LipiFlow for the Treatment of Meibomian Gland Dysfunction and Symptoms: A Randomized Clinical Trial. Clin Ophthalmol. 2020 Feb 12;14:405-418.

3. STATISTICAL CONSIDERATIONS

3.1.General Considerations

Descriptive statistics will be used to summarize continuous variables (number of eyes/patients [N], mean, standard deviation (SD) or standard error of the mean, median, maximum, and minimum) and categorical variables (frequency and percentage) at each assessment timepoint. Statistical comparisons within each group will be performed using appropriate statistical methods or models, and the results such as 95% confidence intervals and p values will be reported. All summary tables will be supported with individual patient and eye data listings. Baseline is defined as the last non-missing observation prior to initiation of study intervention. Analyses will be carried out using SAS software or equivalent statistical software.

Further details will be described in the statistical analysis plan, which will be finalized prior to the study database lock.

3.2.Analysis Populations

- Intent-to-Treat Population: The intent-to-treat (ITT) population includes all randomized patients. Patients will be analyzed as randomized.
- Per Protocol Population: The per protocol (PP) population includes all patients who performed the 4 weeks follow-up visit, completed all the treatments as randomized, and have no major protocol deviations. Patients will be analyzed as treated. The primary analysis population will be the per-protocol (PP) population.
- Safety Population: The safety population will include all treated patients. The safety population will be analyzed for all safety assessments. Patients will be analyzed as treated. A summary table of number (%) of patients in each analysis population/set will be provided.

3.3.Demographic and Baseline Characteristics

Descriptive statistics or frequency tabulation will be provided for demographic and baseline characteristics, such as age, gender, race, and ethnicity.

3.4.Effectiveness Analyses

3.4.1. Primary effectiveness endpoint

- The primary endpoint of change from baseline in the TBUT as rated by a masked rater at 4 weeks will be analyzed for non-inferiority using a margin of 2.5 seconds.
  The primary non-inferiority hypothesis set will be:

Ho: $\mu_{Tixel} - \mu_{Lipiflow} + \delta \leq 0$

HA: $\mu_{Tixel} - \mu_{Lipiflow} + \delta > 0$

where μ_{Tixel} and $\mu_{Lipiflow}$ are the mean TBUT changes from baseline at 4 weeks for Tixel and Lipiflow, respectively and δ=2.5.

A change in TBUT of at least 2.5 seconds in each group will be considered clinically significant.

The difference between Tixel and Lipiflow in changes from baseline in TBUT as rated by a masked rater at 4 weeks will be assessed using a linear mixed-effects model with a random effect for subjects and fixed effect for treatment and baseline score as covariate. The random-effect adjusts the "within-subject correlation" between eyes. Least square means will be estimated by treatment arm from the model. The confidence interval for the estimates will be one-sided and a test value of 2.5 will be used to test non-inferiority.

3.4.2. Secondary effectiveness endpoints

1- OSDI

- The change from baseline at 4 weeks and 12-weeks in the Ocular Surface Disease Index (OSDI) will be calculated along with 95% confidence interval. The Paired T-test or Signed rank test for two means (paired observations) (as is appropriate) will be applied for testing the statistical significance of the changes within each study group.
- The two-sample T-test or Non-parametric Wilcoxon-Mann-Whitney Rank sum test for independent samples (as is appropriate) will be applied for testing the statistical significance of the difference in change from baseline in OSDI at week 4 between the study groups

2- MGS

- The difference between Tixel and Lipiflow in changes from baseline in MGS as rated by a masked rater at 4 weeks and 12 weeks will be assessed using a linear mixed-effects model with a random effect for subjects and fixed effect for treatment and baseline score as covariate. The random-effect adjusts the "within-subject correlation" between eyes. Least square means will be estimated by treatment arm from the model.

3- TBUT

- In 12 weeks, the follow-up visit will be analyzed in the same way as the 4-weeks follow-up visit.

3.4.3. Exploratory Analysis:

- For each follow-up (4 weeks and 12 weeks), if non-inferiority is achieved, the TBUT score change from baseline as rated by a masked rater at the relevant follow will be analyzed for superiority. The hypothesis set will be:
- Ho: $\mu_{Tixel} - \mu_{Lipiflow} \leq 0$
- HA: $\mu_{Tixel} - \mu_{Lipiflow} > 0$

3.5.Handling of Missing data

Every effort will be made to complete follow-up for all patients and avoid missing data, in particular regarding the primary endpoint variable. However, in the event data is missing, multiple imputation will be used. Further details will be provided in the statistical analysis plan.

3.6.Interim Analysis

No interim analyses are planned for this study.

3.7.Sample Size Justification

55 patients will be enrolled in each treatment group.

Sample size rationale:

The sample size calculation is based on the primary efficacy endpoint. Specifically, the sample size calculation was based on the non-inferiority with a 2.5 second margin of the change from baseline in TBUT score as assessed by a masked rater at 4 weeks follow-up for Tixel versus LipiFlow. The expected standard deviation of the difference in change is 4.5 seconds.

The calculation assumes the worst-case scenario that the TBUT of both subjects eyes subjects is completely correlated (r=1).

Sample size justification:

When the sample size in each group is 44, a two group one-sided 0.05 significance level t-test will have 80% power to reject the null hypothesis that the test and standard are not non-inferior (the difference in means, μ_T-μ_L, is 2.5 seconds or farther from zero in the same direction) in favor of the alternative hypothesis that the means of the two groups are non-inferior, assuming that the expected difference in means is 0 and the common standard deviation is 4.5 s.

Assuming a 20% drop-out rate, 55 patients will be enrolled in each group ensuring a sample size of 44 patients per group.

[4 -Dixon et al, 1983; 5 O'Brien et al, 1993]

3.8.Poolability Analysis

Patient demographic characteristics and background variables shall be summarized.

The primary endpoint analysis will be conducted for each investigational site. The justification for pooling all the data to estimate a common effect across study sites requires the homogeneity of response across study sites. An analysis of variance (ANOVA) of the differences will be generated to test whether the investigational sites differ with respect to primary study endpoint (only the site will be included in this analysis model). The test of inhomogeneity of response will be based on a two-sided significance test at the 0.15 level of significance. If the sites differ by this test, then a second analysis will be done including study site and all baseline characteristics that have $P<0.10$ in the analysis of baseline characteristics by site to understand if the imbalance in the primary endpoint between sites is related to an imbalance in baseline characteristics.