STUDY PROTOCOL

Assessment of ther**r**anostic **g**uided rib**o**flavin/UV-A corneal cross-linking for treatment of keratoconus

ACRONYM: ARGO

PROTOCOL NUMBER: RSKC001

PROMOTER & SPONSOR: Regensight srl, Via Livenza 3, 00198 Rome (Italy)

SPONSOR CONTACT: Dr. Marco Lombardo, C.E.O., mlombardo@regensight.com

TRIAL TYPE: PRE-MARKET CLINICAL STUDY

PROTOCOL VERSION: 1.0 - 28/06/2021

REVISION HISTORY:

1.0	First emission

SITES OF INVESTIGATION:

- Azienda Ospedaliera Universitaria Careggi, Università di Firenze
- Azienda Ospedaliera Universitaria Policlinico G. Martino, Università di Messina
- Azienda Ospedaliera Universitaria Mater Domini – Università Magna Graecia di Catanzaro

COORDINATOR SITE OF INVESTIGATION:

- Azienda Ospedaliera Universitaria Mater Domini – Università Magna Graecia di Catanzaro

INVESTIGATIONAL MEDICAL DEVICE: C4V CHROMO4VIS release sw 2.0.0

MANUFACTURER: Regensight srl, Via Livenza n.3, 00198 Rome (Italy)

PROTOCOL APPROVAL and SIGNATURES

Sponsor

I declare that the protocol ARGO (Version 1.0 - 28/06/2021) contains all necessary information required for the conduct of the trial.

Dr. Marco Lombardo – Chief Executive Officer (C.E.O.) 04/10/2021

[redacted] [redacted]

Investigators' Statement

I have carefully read this protocol and agree to conduct the study in accordance with Good Clinical Practice and all applicable government regulations relevant to the use of new and approved therapeutic agents in human subjects.

I agree that Regensight srl, its delegates and Regulatory Authorities have direct access to all study documentation.

I agree to obtain the written Informed Consent from all participating subjects.

I agree to maintain the confidentiality of all information received or developed in connection with this clinical trial.

Prof. Rita Mencucci – Investigator [redacted]

[redacted]

Prof. Anna Maria Roszkowska – Investigator [redacted]

[redacted]

Prof. Vincenzo Scorcia – Investigator [redacted]

[redacted]

Declaration of the Sponsor on the sources and types of financial, material and other support

The Sponsor loans, free-of-charge, the medical devices and the equipment required to perform treatment to the Investigators/Institutions for the entire duration of the trial. The free of charge supply of these materials does not influence the scientific, technical and procedural autonomy of the Investigators and Study staff.
The medical devices used in this Study will be stored and handled according to the applicable Laws.
The equipment will be handle with care and used exclusively in the clinical trial.
The Sponsor will train the investigators on how to use these devices. The Sponsor undertakes the liability insurance required for each subject enrolled in the Study.

Annexes

Annex 1 [redacted]

Annex 2 [redacted]

Annex 3 [redacted]

Annex 3 bis [redacted]

Annex 3 ter [redacted]

Annex 4 [redacted]

Annex 5 [redacted]

Annex 6 [redacted]

Annex 7 [redacted]

Annex 8 [redacted]

Annex 9 [redacted]

Annex 10 [redacted]

Annex 11 [redacted]

Annex 12 [redacted]

Rationale of the Study and Risk/Benefit analysis

Keratoconus is a naturally-occurring ocular condition characterized by progressive thinning and steepening of the central cornea, resulting in corneal optical irregularities with increasing myopia, irregular astigmatism, corneal opacity and consequential loss of visual acuity. Clinical onset of the disease begins in the second decade of life and has significant social impact due to its progression towards vision loss; indeed, keratoconus still represents the primary cause of corneal transplantation in young adults globally.

Riboflavin/UV-A corneal cross-linking is a procedure used to biomechanically stabilize the weak cornea in keratoconus and to slow down or halt the clinical progression of this disease. The procedure has been performed in Europe since 2003, thereafter the procedure has been adopted in eastern countries and United States. The standard corneal cross-linking protocol, also known as *Dresden protocol*, consists in removing the epithelium and administering a riboflavin ophthalmic solution onto the corneal stroma for 30 minutes; afterwards the cornea is irradiated by a UV-A light device using 3mW/cm^2 power density for 30 minutes, with a total delivered energy of 5.4 J/cm^2. According to the systematic review and meta-analysis studies, the standard cross-linking protocol is effective (where efficacy is indicated as stabilization or flattening of the maximum simulated keratometry value, K_{max}) in almost 70% of treated cases, with a failure rate ranging from 8% to 33% one year after surgery. However, epithelial removal is the predisposing factor to the most frequent and major complications of the standard protocol, which include ocular pain, corneal haze, transient corneal edema in virtually all cases, and some severe adverse events, such as corneal infections, corneal melting, and corneal scarring, which may cause vision loss. For this reason, any improvement, which can increase the efficacy and minimize the risks of the gold standard surgical technique is highly desirable. Several transepithelial treatment protocols have been developed in order to minimize risks, nevertheless their clinical efficacy in comparison with the standard protocol still remains object of debate.

Theranostics is an emerging therapeutic paradigm that enables monitoring of image-guided therapy in clinic through the use of a theranostic module that makes use of real-time non-invasive molecular analysis of the tissue being treated to achieve optimal treatment outcomes in the management of disease.

This is a clinical study consisting of a study arm to assess the performance of the theranostic software module (Research Use Only) of a CE marked (CE1936) UV-A medical device, C4V CHROMO4VIS™, in order to validate its use for image-guided corneal cross-linking treatment of keratoconus and corneal ectasia. The study hypothesis is that theranostic-guided riboflavin/UV-A corneal cross-linking with the C4V CHROMO4VIS™ system is safe and can estimate treatment efficacy during operation, regardless of treatment protocol, i.e., either with or without epithelial

removal. The software module is able to measure the concentration of riboflavin into the cornea during treatment and to provide the surgeon with an objective assessment of treatment efficacy. The scope of this study is to validate the theranostic imaging biomarker score by assessing the change of corneal topography K_{max} value at 1-year postoperatively. The 1-year follow-up is long enough to provide scientific evidence of the safety and efficacy of the theranostic UV-A medical device in question. A pre-operative examination will ensure that every interested and willing participant fulfils the inclusion criteria of this study. Post-operative examinations will be carried out after 1 week, 1 month, 3 months, 6 months and 12 months.

The present study can start treatment of subjects only after an Independent Ethical Committee written approval; it is governed by three guiding principles: safety, ethical conduct, and efficiency. Subject safety guides study design by minimizing the number of participants exposed to serious adverse events.

Abbreviations

AE	Adverse Event
CDVA	Corrected Distance Visual Acuity
C4V CHROMO4VIS™	Theranostic UV-A medical device
C4V	Theranostic UV-A medical device acronym
CFR	Code of Federal Regulation
CRF	Case Report Form
CRO	Contract Research Organization
CTA	Clinical Trial Authorization
CV	Curriculum Vitae
CXL	Corneal cross-linking
D	Diopter
EpiOFF	Epithelium off
EpiON	Epithelium on or transepithelial
GCP	Good Clinical Practice
GDPR	General Data Protection Regulation
GMP	Good Manufacturing Practice
ICH	International Council for Harmonization
IEC	Independent Ethical Committee
K_{max}	Maximum Simulated Keratometry
LED	Light Emitting Diode
LogMAR	Logarithmic Minimum Angle of Resolution
RitSight™	0.22% riboflavin eye drop
SAE	Serious Adverse Event
UCVA	Uncorrected Distance Visual Acuity
UV-A	Ultraviolet light A

Table of Contents

Synopsis

Study Population

Ages eligible for study: from 18 years old to 40 years old.

Genders eligible for study: all genders.

Accepts Healthy Volunteers: No.

Inclusion Criteria

Diagnosis of progressive keratoconus.

The criterion to determine progression of keratoconus is based on providing at least one of the following types of evidence:

- at least two Placido disk corneal topography measurements showing at least +1.00 D steepening of the K_{max} value in the last year or longer interval period.
- at least two manifest refraction measurements showing at least -0.50 D change in spherical equivalent refraction in the last year or longer interval period.
- at least two central corneal thickness (CCT) measurements showing at least -10 µm change in in the last year or longer interval period.

Exclusion Criteria

- Anterior corneal curvature steeper than 63 D;
- Corneal thickness thinner than 400 µm;
- Corneal scarring;
- Descemetocele;
- History of herpetic keratitis;
- Concomitant eye diseases;
- Inflammatory eye diseases;
- Glaucoma;
- Cataract;
- Nistagmus;

- Pregnancy;
- Breast feeding.

Ocular history

From each study participant a complete medical and ocular history shall be obtained.

- Family history of keratoconus.
- History of dermatologic pathology.
- History of atopy or allergy.
- History of contact lens wear.
- History of eye rubbing.
- History of medication.

Study objective

The scope of the study is to evaluate a novel modality of riboflavin/UV-A corneal cross-linking based on theranostics aiming at improving predictability of treatment efficacy for better patient care. The objective of the study is to validate the theranostic imaging biomarkers by assessing the mean change of K_{max} value at 12 months after riboflavin/UV-A corneal cross-linking for the treatment of keratoconus.

Study design

Subjects with a diagnosis of progressive keratoconus will be evaluated for suitability as a candidate for riboflavin/UV-A corneal cross-linking. Participants will be evaluated at baseline, day 0 (treatment), day 7, day 30, day 90, day 180 and day 360 after treatment. Informed consent will be obtained from each subject before performance of any required procedures.

Eye examination

Each study participant will undergo at baseline and at each follow-up visit:

- Uncorrected Distance Visual Acuity (UDVA) with ETDRS chart.
- Corrected Distance Visual Acuity (CDVA) with ETDRS chart.
- Manifest Spherical Equivalent Refraction (MSER) with ETDRS chart.

- Maximum Simulated Keratometry (K_{max}) and Central Corneal Thickness (CCT) with combined corneal topography and pachymetry.
- Endothelial Cell Density (ECD) with corneal specular microscopy.
- Slit-lamp examination of the cornea, anterior chamber and lens.

Study arm

One study arm receiving riboflavin/UV-A corneal cross-linking with either standard, Epi-OFF, or transepithelial, Epi-ON, treatment protocol. Only one eye of each participant is designated as the study eye.

Stratification, Allocation and Enrolment

Eligible participants will be stratified with allocation ratio 1:1 into either treatment protocol using a computer-generated stratification plan with blocks. Two different blocks are created, which include eyes with K_{max} steeper or flatter than 54.0 D to allocate patients with comparable baseline K_{max} values in either treatment protocol.

This is a multi-center clinical trial. The stratification code is given to each local site of investigation by the central monitoring site of the Sponsor after the participant has been considered eligible to the study and has signed the informed consent. The enrolment is competitive. The period for enrollment includes the four months after study protocol approval by the IEC of the local site of investigation.

Sample size

This study aims to validate the theranostic imaging biomarkers by assessing the change of maximum keratometry (K_{max}) after corneal cross-linking treatment (CXL). Based upon a systematic literature review, the study hypothesis is that CXL treatment can averagely flattening the K_{max} value by -1.05 D (SD: ±0.80 D) at 1-year after CXL. To this scope, expecting a response rate of 50% of cases that will reach the threshold of -1.05 D of the K_{max} value, a sample size of 42 patients achieves 91% power to detect a difference of 0,2500 between the area under the ROC curve (AUC) under the null hypothesis of 0,6000 and an AUC under the alternative hypothesis of 0,8500 using a two-sided z-test at a significance level of 0,05000. Considering a ≤20% drop-out rate (determined from

systematic literature review on controlled and open clinical studies on standard and epithelium-on CXL procedures), a number of 50 participants (25 per treatment protocol) is allowed to be enrolled in the study.

Products' description

The test articles to use in this study include two CE marked medical devices, such as the investigational one, herein the C4V CHROMO4VIS™ (CE1936) and the 0.22% riboflavin ophthalmic solution, herein the RitSight™ eye drop (CE0477), which must be applied onto the cornea before UV-A irradiation to protect the internal ocular structures from excessive UV-A light radiation.

The C4V CHROMO4VIS™ is a CE marked class IIb electronic UV-A medical device, which delivers ultraviolet light (365 nm wavelength) in a circular area onto the cornea. It is a portable system with articulating arm to allow movement of the system for alignment of the UV-A beam onto the patient's cornea. UV irradiance and irradiation time at the cornea are controlled by an onboard computer system. The medical device is equipped with the theranostic software (for research purpose only) that is the object of validation in this study. The RitSight™ is a CE marked sterile class IIa medical device consisting of a 0.22% riboflavin enriched solution for effective penetration of riboflavin into the corneal tissue to protect the inner ocular structures during UV-A irradiation.

Treatment

Corneal cross-linking is performed with either the corneal epithelium intact or de-epithelialized in patients with progressive keratoconus. The ophthalmic solution *RitSight*™ is applied onto the cornea and its concentration into the cornea monitored by C4V CHROMO4VIS™ system with theranostic software module (v. 2.0). After the pre-set time for application of riboflavin onto the cornea is ended (i.e., 15 min. for epi-off CXL and 20 min. for epi-on CXL protocol), the cornea is illuminated by the C4V CHROMO4VIS™ system using 5.4 J/cm^2 UV-A energy dose (10 mW/cm^2 for 9 minutes).

Primary End Point and Outcome Measure

Time point 12 months: validation of the theranostic imaging biomarkers.

Validation of the combined use of the theranostic imaging biomarkers. True positives, false positives, true negatives and false negatives were evaluated in order to determine the ability of the theranostic imaging biomarkers, including both the *riboflavin score* and *theranostic score*, to predict the propensity of CXL treatment in flattening the K_{max} value (D) by more than 0.1 D at 12 months postoperatively. True positives, false positives, true negatives and false negatives will be evaluated.

Secondary Outcome Measures

- Efficacy: Change of Maximum Keratometry (K_{max}) assessed by corneal topography at 12-months.
- Safety: change of Endothelial Cell Density (ECD) assessed by specular microscopy at 12-months.

Exploratory Outcome Measures

- Change of Uncorrected Distance Visual Acuity (UDVA) assessed by ETDRS at 12-months.
- Change of Corrected Distance Visual Acuity (CDVA) assessed by ETDRS at 12-months.
- Change of Manifest Refraction assessed by ETDRS at 12-months.
- Change of primary and secondary outcome measures in either stratification group at 12-months.

Adverse events

Any adverse event (AE) occurring in a participant enrolled into the clinical trial shall be reported in detail on the CRF according to the ICH Guidelines and the MDCG 2020-10-2 Guidance safety report form. The Medical Dictionary for Regulatory Activities (MedDRA®) is used to code all AEs.

Study Records/Source Documents/Personal Data protection

Adequate records (*Case Report Forms*) will be maintained for the study including subject medical and surgical records, test reports, signed informed forms, and information about subject discontinuation. All original documentation will remain at the investigative sites. Study data that are generated electronically (e.g. corneal topography etc.) will be printed and retained in the study files. A unique identifier will be assigned by the Investigators to each trial subject to protect the subject's identity.

Dropouts/Lost-to-follow-up/withdrawal

Participants may drop out at any time during the study. Based on the scientific literature and considered the 1-year follow-up, a ≤20% dropout may be expected in the study. A total number of 50 participants is therefore allowed to be enrolled in the study.

Statistical Methods

Descriptive statistics will be used to summarize the data: numerical variables will be summarized as mean and standard deviation or median and interquartile range, respectively, according to the distribution of the data; categorical variables will be represented as frequencies and proportions. Student's t-test or Wilcoxon test will be applied to compare continuous variables, while Fisher's exact test or Chi-Squared test will be used to analyze categorical variables. ROC curve analysis will be used to evaluate cut-off values for the theranostic imaging biomarker scores in predicting the propensity of CXL treatment to flatten the K_{max} value at 12-months. The cut-off value for the biomarker scores will be determined by optimizing the Youden index. Moreover, sensitivity, specificity, proportion of correctly classified eyes and positive predictive value (PPV) positive predictive values (PPV) and false negative rate (FNR) and confidence intervals will be calculated.

The study set a minimum threshold of 85% for the theranostic software module's accuracy and precision in predicting the propensity of CXL to halt disease progression (intended as more than 0.1 D K_{max} flattening) at 1 year in the study population (per protocol population).

Statistical significance will be set at 0.05. All the analysis will be performed using the statistical software R (latest version available).

Data Analysis

Data will be summarized using descriptive statistics and graphical presentations.

Numerical variables will be summarized as mean and standard deviation (SD) and as median and interquartile range (IQR). Categorical variables will be represented as frequencies and proportions.

For numerical variables, paired Student t-test or Wilcoxon signed-rank test will be used to compare the distributions preoperatively and 12-months postoperatively. Bonferroni correction will be

applied to analysis of exploratory outcome measures of stratification groups. For all reported AEs, the data shall be presented with events listed in order of decreasing frequency.

Sites of Investigation

- Azienda Ospedaliera Universitaria Careggi, Università di Firenze
- Azienda Ospedaliera Universitaria Policlinico G. Martino, Università di Messina
- Azienda Ospedaliera Universitaria Mater Domini, Università Magna Graecia di Catanzaro

Coordinator Site of Investigation

- Azienda Ospedaliera Universitaria Mater Domini, Università Magna Graecia di Catanzaro

List of principal investigators

Prof.ssa Rita Mencucci, ophthalmologist (UniFI)

Prof.ssa Anna Maria Roszkowska, ophthalmologist (UniME)

Prof. Vincenzo Scorcia, ophthalmologist (UniCZ)

Declaration of the Sponsor on the sources and types of financial, material and other support

The Sponsor has provided the clinical investigators and Site of Investigations with the Protocol (including Case Report Forms), the investigator's brochure, confidentiality, personal data safety and sponsor agreements, and any other documentation, which is necessary to complete the Study.

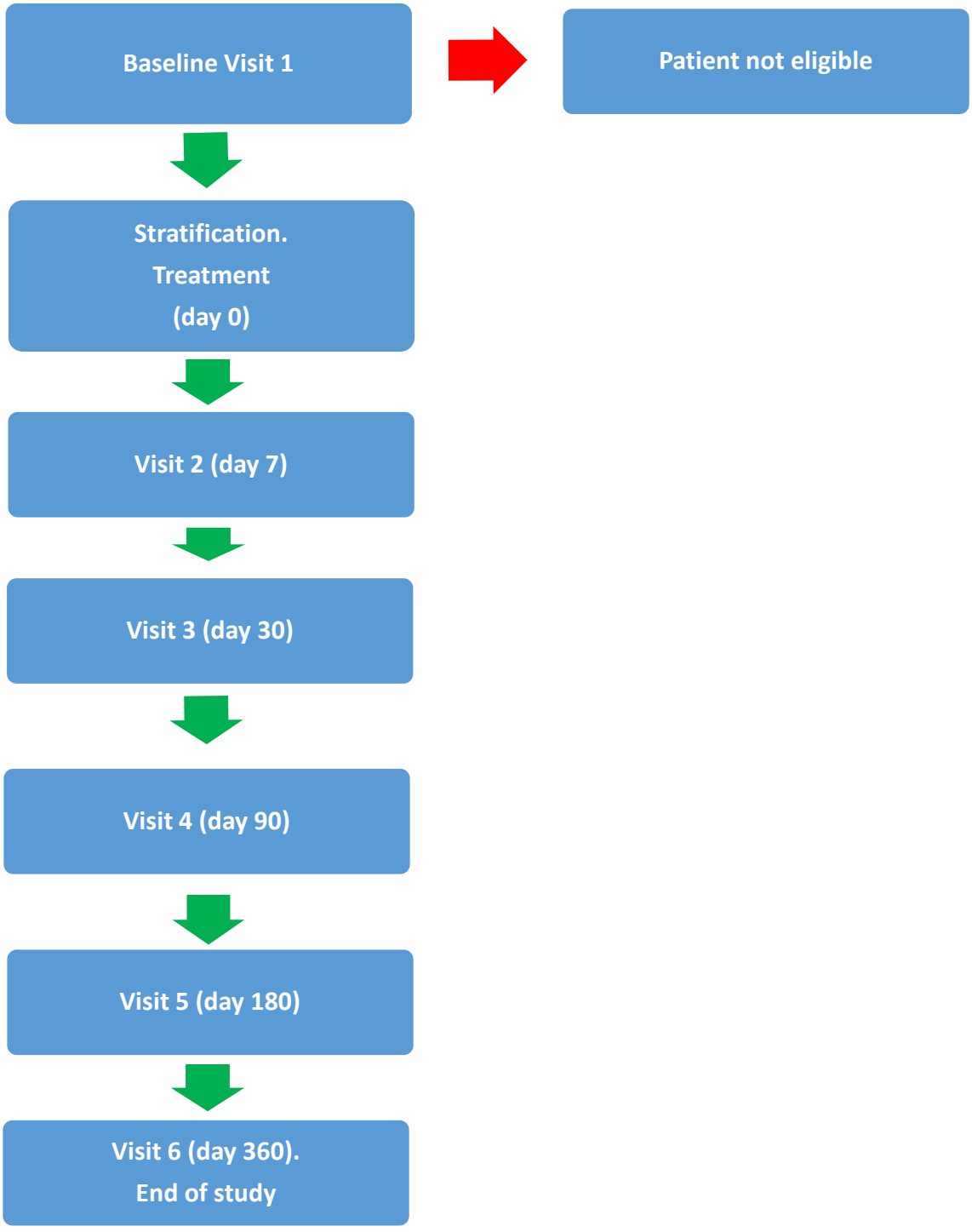
The Sponsor has verified in local audit that each clinical center has a designated clinical area and equipment as well as that the investigators are qualified and experienced to perform the present Study.

The Sponsor loans, free-of-charge, the medical devices and the equipment required to perform treatment to the Investigators/Institutions for the entire duration of the trial. The free of charge supply of these materials does not influence the scientific, technical and procedural autonomy of the Investigators and Study staff. The medical devices used in this Study will be stored and handled according to the applicable Laws. The equipment will be handled with care and used exclusively in the clinical trial. The Sponsor will train the investigators on how to use these devices. The Sponsor undertakes the liability insurance required for each subject enrolled in the Study.

Regulatory aspects and Funding Sources

This clinical investigation is conducted in accordance with the provisions of articles 62 to 80 and Annex XV of the Regulation (EU) 2017/745 as part of the clinical evaluation for conformity assessment purpose of the UV-A theranostic medical device C4V CHROMO4VIS®. The object of the clinical investigation is the validation of the theranostic software module of a CE certified UV-A medical device for the treatment of pathological conditions of the cornea. The device in question conforms to the general safety and performance requirements apart from the aspects covered by the clinical investigation; with regard to those aspects, every precaution has been taken to protect the health and safety of the study participants.

Study protocol overview



Background and State-of-the-Art

Keratoconus is a degenerative corneal disease characterized by tissue weakening and thinning, which lead to corneal bulging (corneal ectasia). The incidence of keratoconus varies between 1:300 and 1:2000 depending upon geographical location (it is more frequent in the south regions of the world). Prevalence of keratoconus is estimated between 0.5% and 3% depending upon geographical location. The etiopathogenesis of keratoconus is multifactorial; familiarity, which is found in 15% - 18% of patients highlights the importance of genetics in the pathological pathway of keratoconus; beyond the genetic heterogeneity of keratoconus, the importance of various environmental factors (UV, atopy), which are able to trigger or accelerate the progression of corneal degeneration, is emphasized in the pathogenesis of the disease.[1,2] The anatomical alterations of the corneal stroma in keratoconus have been correlated to a biomechanical tissue weakening. A number of experimental and clinical studies has shown decreased stiffness and altered viscoelasticity in keratoconus; the main theory is that tissue bulging is caused by inability of the keratoconic cornea to resist the physiologic intraocular pressure levels.

Clinical onset of keratoconus begins in the second or third decade of life and involves a progressive vision loss caused by irregular astigmatism, induced myopia, and in more advanced cases, corneal scarring. Due to the young age of onset, keratoconus may have a significant negative impact on the quality of life of patients. Although contact lenses can provide the majority of patients with adequate visual quality, they are not able to slow down disease progression, which results in progressive vision loss.

Treatment of keratoconus includes the use of eye glasses or contact lenses to improve visual acuity. The surgical treatment of keratoconus includes corneal cross-linking, which aims at stabilizing the structure and tissue biomechanics and halting disease progression. In more advanced cases, corneal transplantation is required to replace the degenerated corneal tissue for restoring visual function. However, corneal transplantation is not a permanent solution to visual disturbance occurring in keratoconus, due to the risk of postoperative complications, such as infections, tissue rejection, elevated and irregular astigmatism, etc. Corneal transplantation at a young age has a 9-16% risk to be replaced in a lifetime, and the risk of rejection is persistent throughout life. For these reasons, a safe and effective intervention, such as corneal cross-linking with riboflavin and UV-A, which could stop disease progression would benefit at large the quality of life of millions of people in the world.

[1] Gordon-Shaag A, Millodot M, Shneor E, Liu Y. The Genetic and Environmental Factors for Keratoconus. Biomed Res Int 2015; 2015: 795738.
[2] Cheung I, McGhee C, Sherwin T. A new perspective on the pathobiology of keratoconus: interplay of stromal wound healing and reactive species-associated processes. Clin Exp Optom 2013; 96: 188-196.

Riboflavin/UV-A corneal cross-linking for the treatment of keratoconus

Riboflavin/UV-A corneal cross-linking (CXL) is a treatment procedure aiming at slowing down or halting disease progression. The treatment consists in the application of riboflavin (vitamin B2) onto the cornea and subsequently in the illumination of the corneal tissue by ultraviolet light (UV-A).[3] The riboflavin has the function to protect the corneal endothelium and inner ocular structures from excessive UV-A light exposure. Authors[4] have demonstrated that CXL induces both a stiffening of the corneal stroma (measured as an increase in the elastic modulus of Young) and a significant decrease of viscoelasticity (measured as reduction of hysteresis). By translating the experimental results to the clinic, the increased corneal stiffness contributes to the stabilization effect on disease progression thanks to a greater tissue resistance to the physiologic intraocular pressure. There are likely other biological mechanisms that may contribute to improving treatment efficacy in the long term, such as the production of collagen proteins and stromal matrix components by the stromal keratocytes, which are regenerated from resident cell population of the posterior stroma after treatment.

The treatment is performed on an outpatient basis under topical anesthesia; after surgery, the patient is prescribed topical medical therapy with antibiotics or antiseptics, anti-inflammatory and lubricant eye drops for a few weeks or months. In some cases, a therapeutic contact lens, which is removed within a few days after surgery, may be applied onto the operated eye.

Riboflavin/UV-A corneal cross-linking has been performed in Europe since 2003, thereafter the procedure has been adopted in eastern countries and United States. Since the first clinical report on CXL published in 2003, over 800 scientific articles have been published discussing the clinical outcomes of the procedure for the management of keratoconus and secondary corneal ectasia.

The standard corneal cross-linking protocol, also known as *Dresden protocol*, consists in removing the epithelium and administering a riboflavin solution onto the corneal stroma for 30 minutes; afterwards the cornea is irradiated by an UV-A light with 3mW/cm^2 power density for 30 minutes, with a total delivered energy of 5.4 J/cm^2. According to the current literature evidences, the standard cross-linking protocol is effective (where efficacy is indicated as stabilization or flattening of the maximum simulated keratometry value, K_{max}) on almost 70% of treated cases, with a failure rate ranging from 8% to 33% one year after surgery. In general, the postoperative clinical course of the *Dresden protocol* shows a reduction in the corrected visual acuity during the first three months after treatment, a worsening of the keratometric topographic indices and a reduction in the central corneal thickness up to six months after treatment. The improvement of all clinical and instrumental

[3] Lombardo M et al. Interaction of ultraviolet light with the cornea: clinical implications for corneal crosslinking. J Cataract Refract Surg 2015; 41(2):446-459.

[4] Beshtawi IM et al. Biomechanical properties of corneal tissue after ultraviolet-A-riboflavin crosslinking. J Cataract Refract Surg 2013; 39(3): 451-462.

biomarkers, in case of therapeutic success, becomes evident by the sixth postoperative month onwards.

The simulated keratometry of the cone apex (K_{max} or maximum keratometry index) is the most used index to demonstrate the progression of keratoconus and the efficacy of the corneal-cross linking. From a thorough literature review assessing clinical data on **6.995 cases treated** by corneal cross-linking with a UV-A medical device for the indication of use, the average change of K_{max} has been **-1.05±0.80 D after 1 year follow-up**, showing that the treatment is effective for stabilizing the cornea, which has been weakened by disease or laser surgery. These clinical data can be considered clinically significant when compared with the **natural history of the disease**, which induces an average K_{max} steepening of **+0.9 D per year**. The bibliographic references revised to determine significance of statistical data in this clinical study can be found in Appendix 1 - Methodology of sample size calculation.

By revising the clinical data on the efficacy of the gold standard protocol, i.e., the *Dresden protocol*, the K_{max} decreases by -1.0 D and -1.7 D in almost 50% of cases at 1-year postoperatively; the corrected visual acuity (CDVA) on average stabilizes (mean change of -0.05 LogMAR in 50% of cases) and the manifest refraction on average changes by +0.50 D (in general, the reduction of the myopic sphere equivalent is between +0.2 D and +0.7 D) in the same period. The central corneal thickness on average remains almost unchanged (+4 µm) compared to the preoperative period at 1 year after treatment. However, epithelial removal is the predisposing factor to the most frequent and major complications of the standard protocol, such as infectious keratitis, sterile corneal infiltrates, corneal scarring, which may cause vision loss.[5] An additional risk factor in the standard protocol is the treatment of corneas with a central corneal thickness lower than 390 µm, due to the risk of inducing phototoxic damage to endothelial structures.

In the last decade, an increasing number of treatment protocols have been developed in order to avoid epithelial removal and to reduce treatment time. Currently, CXL can be performed according to various treatment protocols, which are classified into two main groups, i.e., the protocols that require corneal de-epithelialization (also called EpiOFF) and those that do not require epithelial removal (also called EpiON or transepithelial protocols). Each type of surgical protocol, both EpiOFF and EpiON, in turn may vary according to the method and time of riboflavin application and the method and time of UV-A irradiation of the cornea.

Currently, CXL is well established to treat progressive keratoconus and tens of thousands of treatments are performed yearly worldwide. Half of patients claim to have their quality of life much more improved after corneal cross-linking treatment and the procedure has proven to be a valid

[5] Alio JL et al. Corneal cross-linking and infectious keratitis: a systematic review with a meta-analysis of reported cases. J Ophthalmic Inflamm Infect 2013;3(1):47.

therapy in halting keratoconus progression and drastically reducing the need of keratoplasty for thousands patients worldwide.

Although CXL has been established as primary surgical option to treat progressive keratoconus, there is no current option to provide predictable outcomes and to minimize risk and improve efficacy for either the standard or the transepithelial protocol. The scope of the present clinical study is to assess the performance of a novel modality for riboflavin/UV-A corneal cross-linking using a theranostic UV-A medical device. Theranostics is going to introduce a novel methodology for targeting the precise therapeutical dose of riboflavin and UV-A into the corneal tissue in order to improve predictability of corneal cross-linking for halting disease progression.

Investigational medical device

C4V CHROMO4VIS™ (software version 2.0)

System Description

The C4V CHROMO4VIS™ is an electronic medical device, which delivers ultraviolet light (365 nm wavelength) in a circular spot onto the area of the cornea to treat after a riboflavin ophthalmic solution has been properly applied. Emitted UV irradiance and energy dose are controlled by an on-board computer system.

Major Components

The major components of the C4V CHROMO4VIS system are:

1. Optics head, including the UV-A, blue, yellow-green and orange LED sources, the iris aperture, the camera, Placido disk and an electronic board.
2. Touch screen display with user interface.
3. Stand, articulating arm and serving tray (accessory).
4. Wheeled cart, including the AC/DC power supply and electronic boards.
5. Foot switch.

The major components of the system are described in detail as follows:

1. The optics head houses an electronic board and the UV-A, blue, yellow-green and orange light sources, the iris aperture, the Placido disk and the camera:

2. The treatment parameters (riboflavin dosing, iris aperture and UV-A irradiation) are selected and confirmed through the user interface touch screen display.

3. The C4V CHROMO4VIS system is a portable system with an articulating arm to allow movement of the system in medical areas for treatment of a patient. A serving tray (accessory) may be added to support medical and surgical supplies items.
4. The power supply of C4V CHROMO4VIS system is managed by an electronic board, which provides power through an AC/DC switch power supply (100 Watt/12 Volt output) compliant with IEC60601-1.
5. The treatment parameters can be confirmed by pressing the foot switch.

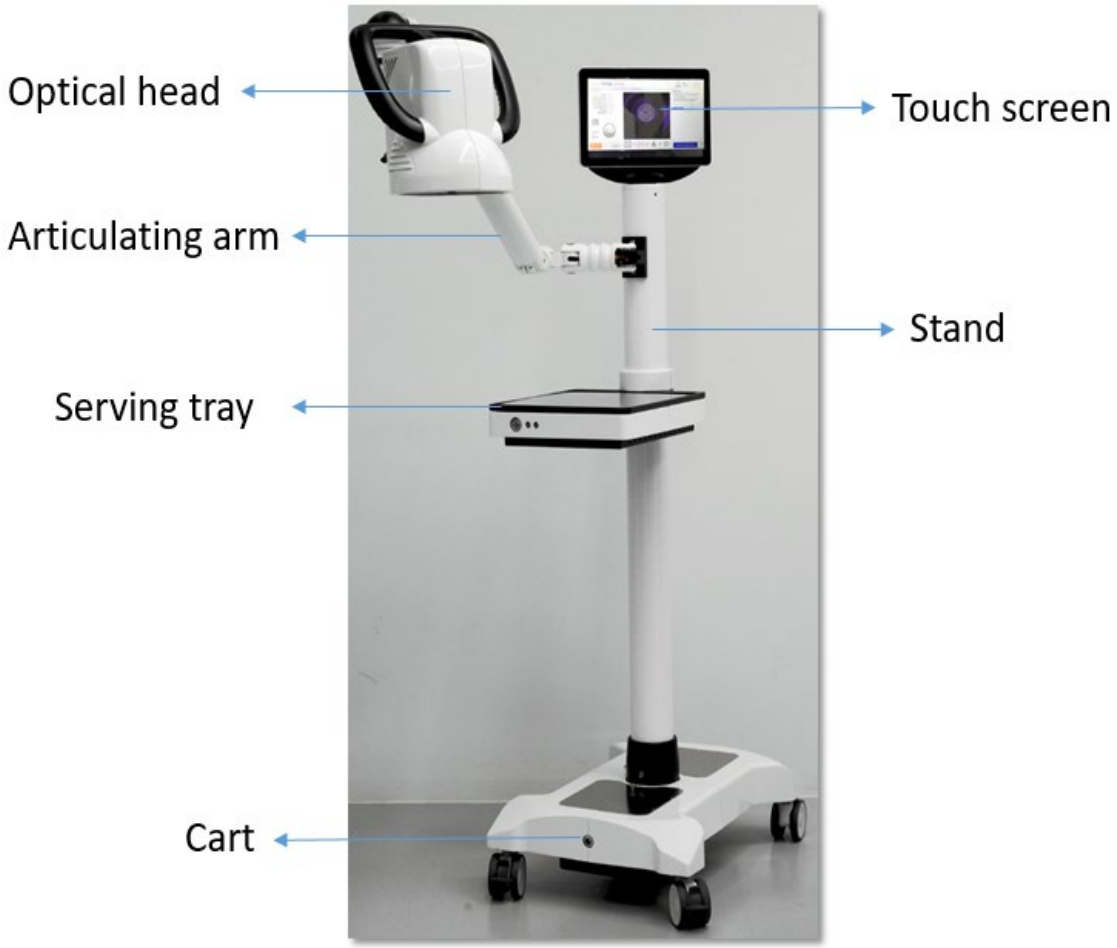


Figure - Overview Illustration of the C4V CHROMO4VIS system.

Technical Features

FEATURE	*TREATMENT*		LIGHT SOURCE
UV irradiance	UV irradiance	[redacted]	[redacted]
	UV exposure time	[redacted]	
	UV emission	[redacted]	
	Measured points	[redacted]	
	Corneal coverage	[redacted]	
	Focusing system	[redacted]	[redacted]
Power monitoring	Double check system	[redacted]	[redacted]

Optical Radiations

FEATURE	LIGHT SOURCE	WAVELENGHT	POWER DENSITY ON EYE
Central fixation LED	[redacted]	[redacted]	[redacted]
Illumination of Placido disk for alignment and focusing	[redacted]	[redacted]	[redacted]
UV-A light source	[redacted]	[redacted]	[redacted]

Environmental Conditions

	IN USE	STORAGE	TRANSPORT
Temperature	[redacted]	[redacted]	[redacted]
Relative humidity	[redacted]	[redacted]	[redacted]
Atmospheric pressure	[redacted]	[redacted]	[redacted]

Electrical Data

Power supply		[redacted]
Power consumption		[redacted]
Fuse	**Type**	[redacted]
	Value	[redacted]

On-Board PC Specifications

Operating system	[redacted]
Processor	[redacted]
RAM	[redacted]
Hard disk	[redacted]
External connections	[redacted]

Touch Screen

Dimension	[redacted]
Ratio	[redacted]
Resolution	[redacted]
Rotation	[redacted]
External connections	[redacted]

System Mechanical Features

Width (max)	[redacted]
Height (max)	[redacted]
Length (max)	[redacted]
Weight	[redacted]
Cart wheels	[redacted]
Degrees of freedom of the balanced arm	[redacted]

Main UV-A medical device operation

- Before any intervention, the C4V CHROMO4VIS must be placed adjacent to the treatment table or chair, away from bright lights, such as positioning in front of windows and the casters must be locked to secure the device's position.
- The device power is turned on by the medical doctor (the "user" or "operator"). The device then checks for startup errors and if the system is starting up correctly, a device calibration is performed.
- The system includes three types of users, such as the Administrator, the Operator (i.e., the surgeon) and the Service. Only the Administrator has database-level privilege and can add/delete surgeons. Therefore, to access patient database, the Operator must be authorized by the Administrator, who has database-level privilege. Once authorized by the Administrator, the Operator can access to the operator main panel and set the treatment parameters for the cornea to treat and the patient's information.
- To begin preparing for treatment, the user enters and/or select the treatment settings:
  - The induction period for the instillation of the 0.22% riboflavin ophthalmic solution in minutes and seconds.
  - Type of UV-A illumination (continuous or pulsed)
  - UV-A energy dose
  - UV-A power irradiance
  - Once the UV-A energy dose and irradiance have been set, the system calculates the UV-A irradiation treatment time in minutes and seconds.
- The user adds the eye to be treated and the corneal parameters (CCT and K_{max}) and confirms to begin treatment.

- The user prepares the eye for treatment.
- The device tracks the induction time and notifies the user that the induction is complete.
- The UV-A treatment is then performed.
- The device tracks the UV-A treatment time, turns off the UV-A and notifies the user when the treatment has been completed.
- Once the treatment has been completed, the user may choose to treat another patient or the device may be powered off.

UV-A irradiation plans

The system is pre-programmed with three pre-set treatment plans:

[redacted]

- Any of the pre-set plans can be edited to change any treatment parameters to create a new pre-set plan that can be saved by pressing the “SAVE” button for future use.
- Any of the pre-set plans can be renamed and then saved for future use.

UV-A irradiation types

The Operator can select the following types of Procedures from the drop down menu of Treatment “Protocol”:

- Epithelium Off protocol (EpiOff).
- Transepithelial protocol (EpiOn).
    - When the Epi On protocol is selected, the option “Enhanced EpiOn” is enabled.
    - By selecting the “Enhanced EpiOn” option, the UV Energy Dose is increased by 10%.

NOTE: After selecting the “EpiOn protocol” and enabling the “Enhanced EpiOn”, the UV Power Density, the UV Delivery Time and the UV-A Energy Dose are automatically increased by 10%.

The Operator can select the following modalities of UV Irradiation from the drop down menu of UV Irradiation “Mode”:

NOTE: the UV Energy (“Dose”) is the product of the UV Power (“Irradiance”) and the UV Irradiation Time. The UV Energy (“Dose”) and the UV Power (“Irradiance”) are adjustable and the calculated UV Irradiation Time is displayed. **After entering the UV Energy Dose and UV Power Density, the UV Delivery Time is automatically calculated and displayed.**

NOTE: the C4V CHROMO4VIS system tracks UV Energy, UV Power, UV Irradiation Time and Total Treatment Time during the treatment. These options are selectable by the user during the treatment plan mode.

The operator may vary the UV Beam Diameter. Use the arrows to increase or decrease the field and enter the desired UV Beam Diameter in the corresponding menu.

Detailed information on the correct use of the C4V CHROMO4VIS system can be found in the User manual v. 1.3 – For Research Use Only (Annex 9).

Contraindications

Conditions that may contraindicate the use of the UV-A medical device include:

- Corneal thickness of less than <370 micrometers.

- Aphakic patients.
- Pregnant and nursing women.
- Eight years old or younger children.

In these situations, the device should not be used because the risk of use may outweigh any possible benefit.

Adverse Events

Corneal cross-linking is a treatment aiming to increase the biochemical stability of the corneal tissue, which has been weakened by disease or surgery, such as keratoconus and corneal ectasia following laser vision correction surgery. The gold standard corneal cross-linking procedure requires the corneal epithelial removal prior to application of a riboflavin ophthalmic solution and subsequent UV-A irradiation of the cornea. The standard procedure has been associated with rare severe adverse events, including infectious keratitis, anterior uveitis and corneal scars (overall incidence <0.2%), which may cause loss of two or more Snellen lines of corrected visual acuity. More recent corneal cross-linking procedures, which do not require for epithelial removal, have not been yet associated with permanent adverse events of the cornea or eye. **The appropriate application of a photo-protective ophthalmic solution prior to UV-A irradiation of the cornea is a fundamental pre-requisite to ensure treatment safety.**

The operator shall ensure to follow all instructions in this manual in order to minimize any possible risk associated with the use of the C4V CHROMO4VIS system and corneal cross-linking procedures.

Precautions

- Contact lens use must be discontinued for at least 3 days before undergoing corneal cross-linking.
- Medical gloves are recommended to be worn.
- Only the patient’s eye that is to be treated should be exposed to the UV radiation.
- Physicians should evaluate the potential benefits in patients with the following conditions:
  - herpes simplex, herpes zoster keratitis;
  - recurrent corneal erosion;
  - epithelial healing disorders;
  - pseudophakic patients.

List of International Standards

The C4V CHROMO4VIS™ system conforms to the followings standards:

[redacted]

Assessing the theranostic software module 2.0

Theranostics is a cutting-edge treatment paradigm. The term “theranostics” refers to the simultaneous integration of therapy and diagnostics and designates a medical solution that provides a precise and personalized therapy for the patient. In this specific case, the medical device C4V CHROMO4VIS 2.0 enables the surgeon to perform the corneal cross-linking procedure by analyzing the images of the corneal tissue to treat.

The C4V CHROMO4VIS 2.0 system provides estimates of the riboflavin concentration in the cornea in real time during treatment, i.e., the riboflavin score, and a predictive index of therapeutic efficacy, i.e. the theranostic score. The purpose of this clinical investigation is to validate the theranostics software v. 2.0 of the medical device in order to determine the threshold of the theranostic imaging biomarker predicting efficacy of the corneal cross-linking procedure, regardless of the CXL treatment protocol.

Theranostic-guided UV-A irradiation plans

For the purpose of this clinical study, the system is pre-programmed with two specific pre-set treatment plans:

- “Theranostic EpiOff CXL” consisting of 15 minutes riboflavin dosing and 9 minutes UV-A irradiation by 10 mW/cm^2.
- “Theranostic EpiOn CXL” consisting of 20 minutes riboflavin dosing and 9 minutes UV-A irradiation by 10 mW/cm^2.

Theranostic-guided CXL operation sequence

- The device power is turned on by the research medical doctor (the “user” or “operator”). The device then checks for startup errors and if the system is starting up correctly, a device calibration is performed.
- The research medical doctor with Administrator user privilege can authorize the Operator user to access patient database and the operator main panel to set the treatment plan for the cornea to treat and the patient’s information.
- Patient data include the key code of the eligible patient to be treated.
- In the treatment plan screen, the Operator adds the eye to be treated and the corneal parameters (CCT and K_{max}) and confirm to begin treatment.
- The Operator prepares the eye for treatment.
- Once the Operator has focused the system onto the eye, a baseline image of the cornea must be acquired by pressing the foot switch for subsequent theranostic real time image analysis.

- During the application phase, apply one drop of riboflavin ophthalmic solution every 20 second directly onto the cornea of the eye to treat.
- During the application phase, the system tracks the application time of riboflavin and notifies the user that the induction is complete. The system provides the Operator with an index estimating the concentration of riboflavin into the cornea.
    - EpiOFF protocol: before measuring the riboflavin concentration into the cornea, dry the corneal surface with a tnt stick.
    - EpiON protocol: before measuring the riboflavin concentration into the cornea, rinse the corneal surface with balanced salt solution.
- The UV-A treatment is then performed. The device tracks the UV-A treatment time, turns off the UV-A and notifies the user when the treatment has been completed. The system provides the Operator with an index estimating the treatment efficacy, i.e., the theranostic imaging biomarker.
    - The operator may choose to rinse the corneal surface with balanced salt solution at 5 minutes interval during UV-A irradiation.
- Once the treatment has been completed, the Operator save the treatment report in a USB stick for printing and adding it to the medical patient record.
- The Operator may choose to treat another eligible patient or the device may be powered off.

Pre-clinical studies on safety of the UV-A medical device

The C4V CHROMO4VIS system has been designed and built in full compliance with EU Directive 93/42/EEC. The medical device has undergone safety testing according to the following standards:

- EMC EN 60601-1-2
- EN 60601-1
- ISO 15004-2:2007
- EN 64271:2010

[redacted]

Results of the emission and immunity testing

Port	Frequency range (MHz)	Test level	Test	Reference standard	Outcome
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

[redacted]

Pre-clinical studies on efficacy of the theranostic UV-A medical device

The efficacy and performance of the UV-A medical device in question has been validated in the laboratory on donor eye bank corneal tissues. The laboratory experiments on the UV-A medical device under evaluation were performed in accordance with the principles of the Helsinki Declaration for the use of human tissues in scientific research. The donor corneal tissues were shipped to the manufacturer's laboratory by the Fondazione Banca degli Occhi del Veneto (Venezia Zelarino) after adequate time of cultivation in special media in order to preserve the integrity of the corneal epithelium and the tissue integrity and transparency.

In the first pre-clinical study, [6] a former group of seven (7) corneal tissues was treated by using the *Dresden protocol*, i.e., with application of riboflavin for 30 minutes and UV-A irradiance of 3 mW/cm^2 for 30 minutes (total 5.4 J/cm^2 energy dose). An additional group of ten (10) corneal tissues was probed by atomic force microscopy (AFM) in order to assess the biomechanical properties of the cornea after corneal cross-linking using the *Dresden protocol*. The scope of the study was to evaluate the performance of the present device in improving the corneal biomechanics strength, measured as increased tissue stiffness (i.e., increased Young's modulus). After corneal cross-linking, the average Young's modulus increased significantly from 1.61±0.53 MPa preoperatively to 2.08±0.72 MPa postoperatively (P=0.001). In summary, the results of the laboratory validation have shown that the UV-A medical device in question is effective in performing corneal cross-linking treatment on human eye corneal tissues by increasing the elastic modulus of the corneal tissue. No alteration of the corneal tissues undergoing corneal cross-linking with the UV-A medical device in question was observed.

In the second pre-clinical study,[7] fourteen (14) eye bank donor corneal tissues were divided into two study groups and treated by the UV-A medical device under evaluation after appropriate application of riboflavin onto the cornea. The first group of seven (7) corneal tissues underwent corneal cross-linking with the *Dresden protocol* (i.e., application of riboflavin for 30 minutes after de-epithelialization and UV-A irradiance of 3 mW/cm^2 for 30 minutes with total 5.4 J/cm^2 energy dose); the second study group of 7 corneal tissues underwent the accelerated corneal cross-linking protocol by using 10 mW/cm^2 UV-A power density for 9 minutes (total 5.4 J/cm^2 energy dose). Pre-irradiation corneal soaking for 30 minutes achieved highly consistent values of intrastromal riboflavin concentration (0.015%±0.003%) between tissues. After UV-A irradiation of the cornea, the mean intrastromal riboflavin concentration was 0.003%±0.001%, with no significant difference between the two UV-A irradiation protocols (P=0.40). In summary, the study showed that the two treatment protocols

[6] Lombardo G et al. Non-invasive optical method for real-time assessment of intracorneal riboflavin concentration and efficacy of corneal cross-linking. J Biophotonics 2018; Jul;11(7):e201800028.

[7] Lombardo M, Lombardo G. Non-invasive and real time assessment of riboflavin consumption in standard and accelerated corneal cross-linking. J Cataract Refract Surg 2019; 45(1):80-86.

performed with the UV-A medical device in question on human eye bank corneal tissues were equivalent in terms of efficacy and performance. No alteration of the corneal tissues undergoing corneal cross-linking with the UV-A medical device in question was observed.

In the third pre-clinical study,[8] ten (10) eye bank corneal tissues were divided into two study groups and treated by the UV-A medical device in question after appropriate application of riboflavin onto the cornea. The first group of five (5) corneal tissues underwent corneal cross-linking with the *Dresden protocol* (i.e., application of riboflavin for 30 minutes onto the cornea after de-epithelialization and UV-A irradiance of 3 mW/cm^2 for 30 minutes with total 5.4 J/cm^2 energy dose); the second study group of 5 corneal tissues underwent transepithelial corneal cross-linking with iontophoresis protocol with epithelium intact (i.e., application of riboflavin for 5 minutes at 1mA/min with corneal iontophoresis followed by UV-A irradiance of 10 mW/cm^2 for 9 minutes with and total 5.4 J/cm^2 energy dose). The application of riboflavin for 30 minutes onto the stroma achieved high corneal riboflavin concentration values (425±77 µg/cm^3); during UV-A irradiation, the stromal riboflavin concentration decreased non linearly in all tissues; at the end of irradiation period, the mean intrastromal riboflavin concentration was 54±29 µg/cm^3, i.e., on average 87% lower than the concentration value obtained at the end of soaking phase. After corneal iontophoresis with intact epithelium, the mean corneal concentration of riboflavin was 195±35 µg/cm^3 (values significantly lower, P=0.001, than conventional corneal soaking); at the end of irradiation period, the mean riboflavin concentration was 31±9 µg/cm^3, i.e., on average 85% lower than the concentration value achieved at the end of soaking phase. In summary, the study showed that UV-A medical device under evaluation can perform either treatment protocols efficiently and that the results of the *Dresden protocol* are superior compared to those of the transepithelial protocol. No alteration of the corneal tissues undergoing corneal cross-linking with the UV-A medical device in question was observed.

In conclusion, the laboratory studies on the UV-A medical device under evaluation have provided sufficient data and information on the safety and performance of the device for the intended purpose of use in an adequate number of human corneal samples. Such data are sufficient to verify that the UV-A medical device is in conformity with all the Essential Requirements pertaining to clinical performance and clinical safety.

[8] Lombardo G, Serrao S, Lombardo M. Comparison between standard and transepithelial corneal cross-linking using a theranostic UV-A device. Graefe's Arch Clin Exp Ophthalmol 2020; 5 Gen E-pub.

Sterile ophthalmic riboflavin solution

Product Description

Ritsight™ is an ophthalmic solution containing riboflavin (0.22%), formulated to allow the penetration of riboflavin into the corneal stroma before ultraviolet (UV-A) irradiation during corneal cross-linking procedures. The purpose of Ritsight is to soak the corneal stroma with riboflavin to constitute a protective barrier against the penetration of UV-A rays beyond the corneal stroma, preserving the internal structures of the eye (corneal endothelium, lens and retina) from damage that light radiation could cause them during corneal cross-linking procedures.

Indications for use

Ritsight™ is indicated to protect the internal ocular structures from excessive exposure to UV-A light rays in the treatment [redacted]

Mode of use

[redacted]

Composition

[redacted]

Description main ingredient	Composition
Riboflavin sodium phosphate	[redacted]

Contraindication

The product is contraindicated in case of known hypersensitivity to components or other substances closely related from a chemical standpoint.

Side effects

Following the instillation of riboflavin ophthalmic solutions, there are no known systemic or local side effects on the ocular structures. Cross-linking treatment is associated with rare severe adverse events, including infectious keratitis, corneal burn and corneal leucoma, the cumulative incidence of which is lower than 0.8%. The incorrect execution of cross-linking treatment, such as insufficient application of riboflavin on the cornea, can increase the risk of onset of side effects, including phototoxic reactions and infections as well as can make the treatment ineffective.

Precautions

- Do not use the product after the expiry date indicated on the package.
- Do not use if the bottle is damaged.
- The medical device must be used immediately after its first opening.
- Each bottle must be used for one treatment only. Any use for multiple treatments may lead to the onset of eye infections.
- Do not use fluorescein on the cornea before instilling the product.
- The medical device is for the exclusive use of the ophthalmologist or under his/her direct control.
- Do not use the medical device outside of the indications for use.
- Do not use in pregnant or nursing women.
- Do not use in 8 years or younger children.
- Store the product away from light and at temperatures between 4° C and 25° C.
- After its first use, the medical device must not be stored but must be disposed of as hospital waste or according to current regulations.

Pre-clinical studies on safety of the 0.22% riboflavin ophthalmic solution

The 0.22% riboflavin ophthalmic solution has undergone biocompatibility testing before CE marking, including:

- Citotoxicity by direct contact according ISO 10993-5:2009.
- Acute Ocular irritation test according ISO 10993-10:2010.
- Delayed hypersensitivity test according ISO 10993-10:2010.

[redacted]

Study Plan

Aim

The scope of the study is to evaluate a novel modality of riboflavin/UV-A corneal cross-linking based on theranostics aiming at improving treatment predictability for better eye care to patients suffering from keratocous. The objective of the study is to validate the combined use of theranostic imaging biomarker scores in predicting CXL treatment efficacy by assessing the change of corneal topography K_{max} value at 12 months after riboflavin/UV-A corneal cross-linking for the treatment of keratoconus.

Type of study and duration

This is a multi-centre clinical study consisting of one study arm with two stratified groups to assess the performance of a new software version (2.0.) of a CE marked class IIb UV-A medical device, C4V CHROMO4VIS (CE1936) for treating keratoconus with corneal cross-linking procedure.
In this study, patients with progressive keratoconus receives a single dose of the ophthalmic solution RitSight™ (CE0477) delivered into the cornea manually for at least 15 minutes and treated by using the C4V CHROMO4VIS™ system with the theranostic software module while they are observed and tested for a period of 12 months after treatment to assess correlation between the theranostic imaging biomarker and the K_{max} flattening.
Follow-up for each participant is twelve months after treatment. The interval is long enough to observe treatment efficacy as well as any effect or complication induced by the innovative corneal cross-linking procedure performed by using the theranostic UV-A medical device in a participant.

Study Participants

The inclusion criteria are: diagnosis of progressive keratoconus. Participants with 18-40 years old are eligible, all genders.
According to the most recent scientific literature[9], the criterion to determine progression of keratoconus is based on providing at least one of the following types of evidence:

- at least two Placido disk corneal topography measurements showing at least +1.00 D steepening of the K_{max} value in the last year or longer interval period.
- at least two central corneal thickness (CCT) measurements showing at least -10 µm change in in the last year or longer interval period.

[9] Ferdi A, et al. Keratoconus Natural Progression: A Systematic Review and Meta-analysis of 11 529 Eyes. Ophthalmology 2019;126:935-945

The exclusion criteria are: corneal apex steeper than 63 D, corneal thickness thinner than 400 µm; corneal scarring; descemetocele; history of herpetic keratitis; concomitant eye diseases; inflammatory eye diseases; glaucoma; cataract; nistagmus; pregnancy; breast feeding.
The study does not accept healthy volunteers.
If both eyes of a participant are eligible, the eye with lower CDVA will be chosen as the study eye.

Study Arm

One study arm receiving either corneal cross-linking treatment after epithelial debridement ("Theranostic Epi-OFF CXL") or transepithelial corneal cross-linking treatment ("Theranostic Epi-ON CXL"). Only one eye of each participant is designated as the study eye.

Discontinuation of contact lens wear

Participants must discontinue the use of contact lenses from seven days before baseline visit to 3 months follow-up; thereafter, they must discontinue the use of contact lenses at least seven days before the remaining follow-up visits at day-180 and day-360.

Outcome Measures and Assessments

Both eyes of each participant will be evaluated at baseline (i.e., before treatment) to assess eligibility; only one eye for each participant will be treated by corneal cross-linking ("study eye"). The study eye of each recruited subject will be assessed to investigate the primary and secondary outcome measures.
Assessment of the novel theranostic-guided riboflavin/UV-A corneal cross-linking treatment modality will be determined objectively by a comprehensive eye examination, measurement of corneal topography and corneal endothelial microscopy.
Follow-up assessments will be performed on day 7, 30, 90, 180 and 360 after treatment.
At baseline (assessment of both eyes) and all the subsequent follow-up visits (assessment of "study eye" only) each subject recruited in the study will undergo the following examinations:

1) Uncorrected Distance Visual Acuity (UDVA) tested using the ETDRS chart.
2) Corrected Distance Visual Acuity (CDVA) tested using the ETDRS chart.
3) Manifest Spherical Equivalent Refraction (MSER) tested with ETDRS chart.
4) Measurement of corneal curvature (K_{max}, K_{steep}, K_{flat}) and corneal thickness (CCT) using a combined Placido-disk computerized corneal topography.
5) Measurement of endothelial cell density (ECD) using the corneal specular microscopy.
6) Slit lamp bio-microscopy of the ocular surface and the anterior segment of the eye.

At baseline (assessment of both eyes) and at last follow-up visit (12-months), each subject recruited in the study will undergo also ocular tonometry and indirect ophthalmoscopy.
The study visits and the battery of tests to be performed at each visit are fully described in next section.
The investigators may opt to perform additional, no-contact, instrumental testing, as a part of their standard of care practice in patients with keratoconus, which are not due for protocol. A rationale for performing additional instrumental testing in participants shall be added in the corresponding box of the CRF (Annex 1).

Study Visits

Each participant will undergo treatment and six study visits. A tabulated summary of the study visits for study groups including the assessments to be performed at each visit is summarized below:

Time	**baseline**	**day 0**	**day 7**	**day 30**	**day 90**	**day 180**	**day 360**
Description	***Visit 1***	***Treatment***	***Visit 2***	***Visit 3***	***Visit 4***	***Visit 5***	***Visit 6***
Informed consent	X						
Medical history	X						
UDVA	X		X	X	X	X	X
CDVA	X		X	X	X	X	X
Manifest Refraction	X		X	X	X	X	X
Corneal Topography/Pachymetry	X		X	X	X	X	X
Corneal Specular Microscopy	X		X	X	X	X	X
Slit Lamp Microscopy	X		X	X	X	X	X
Ocular Tonometry	X						X
Dilated fundus Examination	X						X
Theranostic data		X					
Adverse Events		X	X	X	X	X	X

Visit 1 – Baseline

Potential candidates will undergo a complete eye examination to determine their eligibility to the study. Before any test is conducted, the participants will be given a brief explanation of the study (including the number and time of visits to be performed).
The baseline visit will provide the confirmation that the participant is eligible for the inclusion in the study and the full medical history will be collected. Baseline measurements will be performed in the following order:

1. Corneal curvature and corneal thickness measurements using the computerized corneal topography device.
2. Endothelial cell density measurement using the specular microscopy.
3. Uncorrected Distance Visual Acuity, measured with ETDRS chart at a test distance of 4 m.
4. Corrected Distance Visual Acuity, measured with ETDRS chart at a test distance of 4 m.
5. Manifest Spherical Equivalent Refraction with ETDRS chart at a test distance of 4 m.
6. Slit lamp bio-microscopy of the ocular surface and the anterior segment of the eye.
7. Intra-ocular pressure (IOP) measurement.
8. Dilated fundus examination.

At the end of the baseline assessment, eligible participants shall the informed consent form (Annex 3) and will be assigned an individual stratification number according to the stratification protocol described in the corresponding section of this study protocol.
Participants will be also reminded to bring sunglasses the day of surgery.

Treatment (day 0)

Participants will receive theranostic-guided cross-linking treatment according to the RUO User Manual and the Investigator's Brochure instructions and the following section "Treatment".
After treatment, each participant will receive medical therapy and relative instructions (Annex 3).

NOTE: To provide standard medical care, the participants treated by EpiOFF theranostic-guided CXL will be visited at day 3 to discontinue the use of contact lens in the treated eye.

Visit 2, 3, 4 and 5 – Follow up (day 7, day 30, day 90 and day 180 respectively)

These visits will provide follow-up measurements, which will be performed in the following order:

1. Corneal curvature and corneal thickness measurements using the computerized corneal topography device.
2. Endothelial cell density measurements using the specular microscopy.
3. Uncorrected Distance Visual Acuity, measured with ETDRS chart at a test distance of 4 m.
4. Corrected Distance Visual Acuity, measured with ETDRS chart at a test distance of 4 m.
5. Manifest Spherical Equivalent Refraction with ETDRS chart at a test distance of 4 m.
6. Slit lamp bio-microscopy of the ocular surface and the anterior segment of the eye.

Any adverse event occurring between the previous and the current visit will be recorded.

A ±2 working day tolerance window will be allowed for visit 2; a ±4 days tolerance window will be allowed for visit 3; a ±10 days tolerance window will be allowed for visit 4 and a ±14 days tolerance window will be allowed for visit 5.

Visit 6 – Follow up (day 360)

This visit will provide last follow-up measurements, which will be performed in the following order:

1. Corneal curvature and corneal thickness measurements using the computerized corneal topography device.
2. Endothelial cell density measurements using the specular microscopy.
3. Uncorrected Distance Visual Acuity, measured with ETDRS chart at a test distance of 4 m.
4. Corrected Distance Visual Acuity, measured with ETDRS chart at a test distance of 4 m.
5. Manifest Spherical Equivalent Refraction with ETDRS chart at a test distance of 4 m.
6. Slit lamp bio-microscopy of the ocular surface and the anterior segment of the eye.
7. Intra-ocular pressure (IOP) measurement.
8. Dilated fundus examination.

Any adverse event occurring between the previous and the current visit will be recorded.

A ±21 working days tolerance window will be allowed for visit 6, which will be the last study visit for all participants enrolled in the study.

Dropouts

Participants may drop out at any time during the study. The list of drop out (Annex 4) shall include information on participants who drop out from the study.

Treatment

In this clinical study, the innovative corneal cross-linking procedure will be performed using the theranostic software module of the C4V CHROMO4VIS™ system in all participants. Participants will receive a single dose of the 0.22% riboflavin ophthalmic solution, RitSight™. Application of the riboflavin eye drop will be done for at least 15 minutes in each participant, specifically 15 minutes for the EpiOFF CXL treatment and 20 minutes for the EpiON CXL treatment. Estimates of riboflavin concentration into the cornea will be monitored by the C4V CHROMO4VIS™ system during the dosing phase of treatment. Upon reaching a minimum threshold concentration into the cornea, defined by the C4V CHROMO4VIS™ system, the system provides the Operator the access to the UV-A irradiation of the cornea with 5.4 J/cm^2 total energy dose and 7.00 mm light beam diameter in all participants.

Patient data: in order to anonymize patient data, the user shall code name using the stratification code provided by the Sponsor at the time of enrolment.

Treatment settings are pre-set for both treatment protocols that are object of investigation in the present clinical study:

Investigational theranostic-guided EpiOFF protocol	**Investigational theranostic-guided EpiON protocol**
- Riboflavin dosing mode: manual - Riboflavin dosing time: 15 minutes - UV-A irradiation mode: continuous. - UV-A irradiation power: 10 mW/cm^2 - UV-A irradiation time: 9 min. - UV-A energy dose: 5.4 J/cm^2 - Beam aperture: 7 mm	- Riboflavin dosing mode: manual - Riboflavin dosing time: 20 minutes - UV-A irradiation mode: continuous. - UV-A irradiation power: 10 mW/cm^2 - UV-A irradiation time: 9 min. - UV-A energy dose: 5.4 J/cm^2 - Beam aperture: 7 mm

The riboflavin/UV-A corneal cross-linking protocol that shall be used in this study is described as follows:

- the C4V CHROMO4VIS™ system must be checked by the user before each use;
- the C4V CHROMO4VIS™ system power is turned on by the user. The device then checks for startup errors and if the system is starting up correctly, a device calibration is performed;
- to begin preparing for treatment, the user select the treatment settings:
  - Induction period for the instillation of the ophthalmic solution *RitSight*: manual.
  - Type of UV-A illumination: continuous.
  - UV-A energy dose: 5.4 J/cm^2.

  - UV-A power irradiance: 10 mW/cm^2.
  - Once the UV-A energy dose and irradiance have been set, the system calculated the UV-A irradiation treatment time in minutes and seconds;
- the user adds the key code of the eligible patient to be treated in the “patient database screen” of the system;
- the user add the eye to be treated and the corneal parameters (CCT and K_{max}) in the “treatment plan screen” of the system and confirm to begin treatment;
- the user prepares the patient’s eye for treatment (numbing eye drops and eye speculum) and access the “treatment screen” of the C4V CHROMO4VIS™ system;
- the user applies the riboflavin ophthalmic solution onto the cornea; the user applies one drop of riboflavin every 20 seconds directly onto the cornea of the eye to treat;
- the user must dry (in EpiOFF protocol) or rinse with balanced salt solution (in EpiON protocol) the excess of riboflavin onto the cornea before measuring its concentration by pressing the foot switch;
- the C4V CHROMO4VIS™ system tracks the riboflavin application time and notifies the user that the induction is complete;
- the user selects to proceed to perform UV-A treatment in the “treatment screen” of the C4V CHROMO4VIS™ system;
- the user may choose to rinse with a drop of balanced salt solution the corneal surface during UV-A irradiation once at 5 minutes interval.
- the C4V CHROMO4VIS™ system tracks the UV-A treatment time, turns off the UV-A and notifies the user when the treatment has been completed;
- the user saves the treatment report in an USB stick;
- the user may choose to treat another eligible patient or the C4V CHROMO4VIS™ system may be powered off.

Detailed information on the treatment procedure can be found in the User Manual – For Research Use Only (Annex 9), including the pictures of the wizard screens.

Patient Counseling Information

Patients shall be advised not to rub their eyes for the first week after their procedure.
Patients may be sensitive to light and have a foreign body sensation. Patients shall be advised that there may be discomfort in the treated eye and that sunglasses may help with light sensitivity.
If patients experience severe pain in the eye or any sudden decrease in their vision, they shall be advised to contact the Principal Investigator of the Site of Investigation immediately.

If the bandage contact lens that is placed on the patient's eye on the day of treatment falls out or becomes dislodged, the patient shall be advised not to replace it and to contact the Principal Investigator of the Site of Investigation immediately.

Study Product Packaging and Storage

The Site of Investigation will receive an UV-A medical device and enough bottles of the RitSight ophthalmic solution to treat all the planned participants. Each Site will receive at least 20 bottles of RitSight™. The carton box of each bottle will be labelled with a white printed label bearing the following information in English:

- **For Clinical Trial Use Only**
- **Study code:** RSKC001
- **Acronym**: ARGO
- **Sponsor:** Regensight srl

The participant box shall be stored in a locked cabinet/locked room at the Site of Investigation's operating room between 4°C to 25°C. Only the principal investigator of the Site will have access to these products during the course of the study. He/She will annotate the stratification number for that participant in the respective CRF.

Test Article Accountability

The principal investigator of the Sites of Investigation will maintain accurate records of receipt of all study products, including dates of receipt. In addition, accurate records will be kept regarding when the study product (participant kit) is dispensed and used by each patient in the study. Reasons for departure from the expected dispensing regimen or dispensing of an extra kit must also be recorded. At the completion of the study, to satisfy regulatory requirements regarding accountability, all study products records will be reconciled.

Study assessments

Demographic Data/Medical History

Key-coded personal details, gender, age, details of general health and eye health and current medication.

Efficacy Assessments

Efficacy data will be identified by using objective and subjective methodologies, as described in the following:

- Measurement of **corneal curvature** with Placido disk topographer will assess changes of the K_{max} after treatment.

Safety Assessments

Safety data will be identified by using objective methodologies, as described in the following:

- Measurement of **Corrected Distance Visual Acuity** (CDVA) with ETDRS chart at a test distance of 4 m will assess changes of the CDVA after treatment.
- Measurement of **corneal endothelial cell density** (ECD) with specular microscopy will assess will assess changes of the ECD after treatment.
- **Slit lamp biomicroscopy** will assess integrity of the cornea and anterior segment of the eye.

Primary outcome measure

Validation of the Theranostic Imaging Biomarker [Time Frame: 12-months]

- Validation of the combined use of the theranostic imaging biomarkers. True positives, false positives, true negatives and false negatives were evaluated in order to determine the ability of the theranostic imaging biomarkers, including both the *riboflavin score* and *theranostic score*, to predict the propensity of CXL treatment in flattening the K_{max} value (D) by more than 0.1 D at 12 months postoperatively.

Theranostics: confirmation of the theranostic imaging biomarker will be provided at the end of treatment procedure. The theranostic data, which will be recorded during treatment procedure, will be exported to a computer for subsequent analysis with 12-months data.

The accuracy and precision (95% CI) of the combined use of the theranostic imaging biomarkers to predict CXL treatment outcome were determined by calculating the proportion of correctly classified eyes and the positive predictive value (PPV) respectively. The miss rate, which is the probability that a true positive is missed by the theranostic methodology, was determined by calculating the false negative rate (FNR).

The study sets a minimum threshold of 85% for the theranostic software module's accuracy and precision in predicting the propensity of CXL to halt disease progression (intended as more than 0.1 D Kmax flattening) at 1 year in the study population, who participated and completed at least 66% of all the visits (i.e., 4 of 6 total visits) and the last at 12 months (i.e., Per Protocol Population;

Secondary outcome measures

Efficacy [Time Frame: 12-months]

- Change of Maximum Keratometry index assessed by corneal topography at 12-months.

Corneal topography: measurement of maximum simulated keratometry (K_{max}, D) will be done by using Placido disk corneal topography. The investigator will record the K_{max} value at each visit and will fill the corresponding CRF. This test is performed to provide evidence of corneal curvature change after treatment.

Safety [Time Frame: 12-months]

- Change of Endothelial Cell Density assessed by specular microscopy at 12-months.

Corneal specular microscopy: endothelial cell density (ECD, cell/mm^2) count will be done by using corneal specular microscopy. The investigator will record the ECD at each visit and will fill the corresponding CRF. This test is performed to provide evidence of corneal cells integrity after treatment.

Other outcome measures

Efficacy [Time Frame: 12-months]

- Change of Manifest Refraction assessed by ETDRS at 12-months.

Manifest Refraction: manifest refraction will be determined using a standard ETDRS chart under photopic conditions (with the luminance of the test at 85 cd/m^2) at a test distance of 4 m. The Manifest Refraction will be assessed by using trial lenses and will be expressed in diopters (D). This test is performed to provide evidence of optical focusing properties change after treatment.

- Change of Best-Spectacle Corrected Visual Acuity (CDVA) at 12-months.

Corrected Distance Visual Acuity (CDVA): CDVA will be determined using a standard "Early Treatment Diabetic Retinopathy Study" (ETDRS) chart under photopic conditions (with the luminance of the test at 85 cd/m^2) at a test distance of 4 m. The CDVA will be expressed in LogMAR. The ETDRS test incorporates specific design criteria to make it more accurate than the Snellen or Sloan acuity tests. These include:

- Same number of letters per row (five letters per row);

- Equal spacing of the rows on a log scale (the rows are separated by 0.1. log unit);
- Equal spacing of the letters on a log scale;
- Individual rows balanced for letter difficulty.

The patient starts are the top of the chart and begins to read down the chart. The patient reads down the chart until he or she reaches a row where a minimum of three letters on a line cannot be read. The patient is scored by how many letters could be correctly identified and by the LogMAR of the line with ≥3 letters he/she is able to read.

- Change of Unorrected Visual Acuity (CDVA) at 12-months.

Uncorrected Distance Visual Acuity (UDVA): UDVA will be determined using a standard "Early Treatment Diabetic Retinopathy Study" (ETDRS) chart under photopic conditions (with the luminance of the test at 85 cd/m^2) at a test distance of 4 m. The UDVA will be expressed in LogMAR. The ETDRS test incorporates specific design criteria to make it more accurate than the Snellen or Sloan acuity tests. These include:

- Same number of letters per row (five letters per row);
- Equal spacing of the rows on a log scale (the rows are separated by 0.1. log unit);
- Equal spacing of the letters on a log scale;
- Individual rows balanced for letter difficulty.

The patient starts are the top of the chart and begins to read down the chart. The patient reads down the chart until he or she reaches a row where a minimum of three letters on a line cannot be read. The patient is scored by how many letters could be correctly identified and by the LogMAR of the line with ≥3 letters he/she is able to read.

Safety [Time Frame: 12-months]

- Change of Central Corneal Thickness assessed by corneal pachymetry at 12-months.
- **Corneal pachymetry:** measurement of central corneal thickness (CCT, µm) will be done by using combined corneal topography/pachymetry device. The investigator will record the CCT value at each visit and will fill the corresponding CRF. This test is performed to provide evidence of corneal thickness change after treatment.

Adverse Events

Reporting adverse events

The Principal Investigator of the Site of Investigation is responsible for reporting all adverse events (AEs) that are observed or reported during the study, regardless of their relationship to study treatment or their clinical significance.

An AE is defined as any untoward medical occurrence in a participant enrolled into this study regardless of its causal relationship to study treatment. Participants will be instructed to contact the

principal investigator of the Site of Investigation at any time if any symptoms develop. A treatment-emergent AE is defined as any event not present prior to exposure to study product or any event already present that worsens in either intensity or frequency following exposure to test medication.
All AEs that occur during the study must be reported in detail on the CRF and followed to satisfactory resolution or until the principal investigator of the Site of Investigation deems the event to be chronic or the participant to be stable. The description of the AE will include the onset date, duration, date of resolution, severity, seriousness, etiology, and the likelihood of relationship of the AE to study treatment.
A serious adverse event (SAE) is defined as any event that results in death, is immediately life threatening, requires inpatient hospitalization or prolongation of existing hospitalization, results in persistent or significant disability/incapacity, is a congenital anomaly/birth defect. Important medical events that may not result in death, be life threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias, or convulsions that do not result in participant hospitalization.

Eliciting Adverse Event Information

Adverse events will be assessed from the time the participant receives the treatment until exit from the study. At every study visit, participants will be asked a standard question to elicit any medically related changes in their well-being. They will also be asked if they have been hospitalized, had any accidents, used any new medications, or changed concomitant medication regimens (both prescription and over-the-counter medications). In addition to participant observations, AEs will be documented from any data collected on the AE CRF or other documents that are relevant to participant safety.

Adverse Event Reporting

All AEs reported or observed during the study will be recorded on the AE CRF according to the ICH Guidelines. Information to be collected includes dosage, type of event, time of onset, investigator-specified assessment of severity and relationship to study Treatment, time of resolution of the event, seriousness, any required treatment or evaluations, and outcome. Adverse events resulting from concurrent illnesses, reactions to concurrent illnesses, reactions to concurrent medications, or progression of disease states must also be reported. All AEs will be followed to adequate resolution.

Medical Dictionary for Regulatory Activities (MedDRA®) will be used to code all AEs. The As will be reported in the CRF according to the mdcg 2020-10-2 - Guidance safety report form.
Any medical condition that is present at the time that the participant is screened but does not deteriorate should not be reported as an AE. However, if it deteriorates at any time during the study, it should be recorded as an AE.

Assessment of Causality

The investigator's assessment of an AE's relationship to study Treatment is part of the documentation process, but it is not a factor in determining what is or is not reported in the study. If there is any doubt as to whether a clinical observation is an AE, the event should be reported.
The relationship or association of the test article in causing or contributing to the AE will be characterized using the following classification and criteria:

Unrelated: This relationship suggests that there is no association between the study treatment and the reported event.

Possible: This relationship suggests that treatment with the test article caused or contributed to the AE, i.e., the event follows a reasonable temporal sequence from the time of treatment administration and/or follows a known response pattern to the test article, but could also have been produced by other factors.

Probable: This relationship suggests that a reasonable temporal sequence of the event with treatment administration exists and, based upon the known action of the treatment, known or previously reported adverse reactions to the treatment, or judgment based on the investigator's clinical experience, the association of the event with the study medication seems likely.

Definite (Causal): This relationship suggests that a definite causal relationship exists between the Treatment administration and the AE, and other conditions (concurrent illness, progression/expression of disease state, or concurrent medication reaction) do not appear to explain the event.

Assessment of Severity

The intensity of the AE will be rated as mild, moderate, or severe using the following criteria:

Mild: These events require minimal or no treatment and do not interfere with the participant's daily activities.

Moderate: These events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.

Severe: These events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually incapacitating.

Changes in the severity of an AE should be documented to allow an assessment of the duration of the event at each level of intensity to be performed. Adverse events characterized as intermittent require documentation of onset and duration of each episode.

Action taken to Adverse Event

The action taken regarding the study treatment (corneal cross-linking) must be clarified in the CRF as follows:

- None.
- Study product not applied.
- Unknown.

Adverse Event treatment required

The need for any treatment to solve the adverse event must be clarified in the CRF as follows:

- None (other than protocol therapy).
- Drug therapy.
- Non-drug therapy.
- Hospitalization.

Outcome of Adverse Event

The outcome of an event will be classified as follows:

- Recovered.
- Recovered with sequelae.
- Ongoing.
- Fatal.
- Unknown/Lost to follow-up.

Serious Adverse Event Reporting

Any AE considered serious by the principal investigator of the Site of Investigation must be reported to IEC and the Sponsor within 24 hours from the time site personnel first learn about the event and during normal business CET hours.

A written report must be submitted within 24 hours of the initial reporting to the IEC and the Sponsor and should consist of the Serious Adverse Event Report Form, accompanied by the following CRF

pages: the demographics page(s), the medical history page(s), the AE page(s) and the concomitant medications page(s). If the participant is hospitalized because of or during the course of an SAE, then a scanned copy of the hospital discharge summary should be sent by email to Sponsor as soon as it becomes available. Withdrawal from the study and all therapeutic measures will be at the discretion of the principal investigator. All SAEs (related or unknown relationship to the study Treatment) will be followed until satisfactory resolution or until the principal investigator or investigator deems the event to be chronic or the participant to be stable.

The Principal Investigator of Site of Investigation will be responsible for telephone or fax reporting of any SAE to the IEC. Site will notify the IEC by telephone or fax transmission of any unexpected, fatal, or life-threatening experience (expedited report) associated with the use of the study Treatment as soon as possible but no later than 7 calendar days after the initial receipt of the information. Initial notification will be followed by a written report within 15 calendar days. For unexpected events associated with the use of the study Treatment, Sponsor/CRO will notify the IEC as soon as possible, but no later than 15 days of the initial receipt of information. The principal investigator is responsible for informing the Ethical Committee. Copies of SAE correspondence with the principal investigator or investigators, governing authorities, ethics committees, and the sponsor must be submitted to Sponsor/CRO for filing.

Adverse Event Monitoring

A participant experiencing any AE or SAE will receive treatment and follow-up evaluations by the principal investigator of the Site of Investigation or will be referred to another appropriate physician for treatment and follow-up. All AEs, whether serious or non-serious, should be followed to resolution or until the AE is determined by the investigator not to be clinically significant.

List of adverse events reported in the specialized literature after CXL treatment

Summary of adverse events on total 9607 cases treated by CXL procedures (ref. bibliography)				
N	***Type of adverse event***	***Intensity of adverse event***	***Cases (n.)***	***Incidence (%)***
1	Bacterial keratitis	Severe	59	0.61%
2	Herpetic keratitis		5	0.05%
3	Corneal burn		1	0.01%
4	Corneal scarring		51	0.53%
5	CDVA loss (>2 ETDRS lines)		13	0.13%

6	Persistent corneal haze	Moderate	12	0.12%
7	Corneal infiltrates		54	0.56%
8	Epithelial ingrowth under LASIK flap		1	0.01%
9	Eyelid eczema	Mild	1	0.01%
10	Transient eye pain		18	0.19%
11	Transient ocular itching		1	0.01%
12	Transient visual fogging		16	0.17%
13	Dry eye		6	0.06%
14	Conjunctival hyperemia		10	0.10%
15	Transient corneal haze		499	5.19%
16	Transient corneal edema		74	0.77%

The table above shows the adverse events reported in studies of eyes treated both by Epi-Off and Epi-On corneal cross-linking procedures. In these studies, the majority of AEs generally developed in the short-term. The percentage of AEs that resolved by 3 months study visit was 98%. The most common AEs (i.e., ≥10%) were corneal epithelium defect, corneal opacity, corneal edema/striae, eye pain, and punctate keratitis. Most of these events represented sequelae following corneal epithelial debridement. Indeed, the Epi-Off corneal cross-linking procedure involves removal of the corneal epithelial layer, which is expected to result in reduction of vision due to the injury to the epithelium. The reduction in visual function and other adverse reactions generally resolve with the healing of the epithelium by the first week after treatment, though visual recovery to baseline values may take up to 3 months after treatment.

Treatment strategy for adverse events

Any adverse event is monitored in order to respond promptly and adequately to each of them. Since all participants will administer antibiotic/disinfectant eye drops and lubricant eye drops immediately after dosing and treatment, the majority of adverse event is managed preventively according to the standard of care after corneal cross-linking. The use of anti-inflammatory eye drops may be required in the case of moderate or severe ocular surface inflammation in a participant. The use of paracetamol pills may be used in the event of unacceptable headache in a participant.

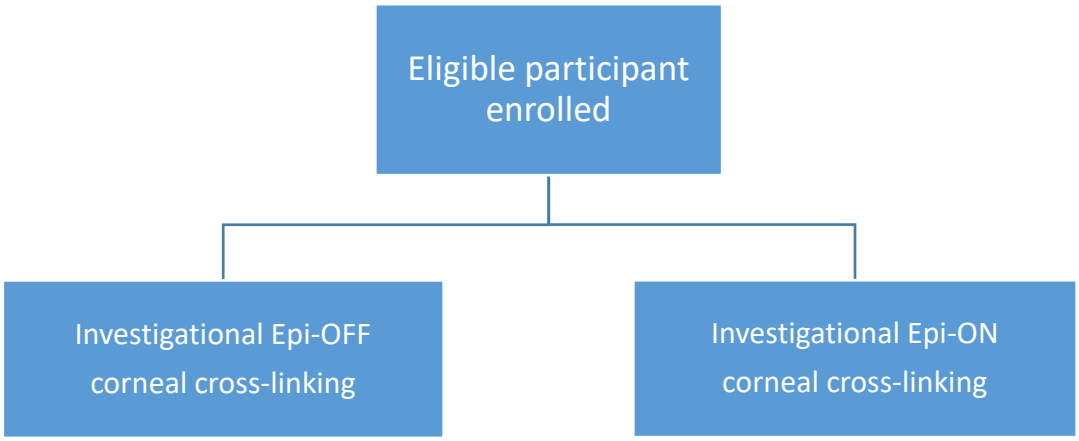
Statistical Considerations and Data Analysis

Stratification, Allocation and Enrolment

Eligible participants will be stratified with allocation ratio 1:1 into either theranostic-guided CXL treatment protocol using a computer-generated stratification plan with blocks. Two different blocks are created, which include eyes with K_{max} steeper or flatter than 54.0 D to stratify patients with comparable baseline K_{max} values in either theranostic-guided CXL treatment protocol.

This is a multi-center clinical trial. The stratification code is given to each local site of investigation by the central monitoring site of the Sponsor after the participant has been considered eligible to the study and has signed the informed consent. The enrolment is competitive. The period for enrollment includes the four months after study protocol approval by the IEC of the local site of investigation.

Synopsis on disposition of participants



Sample size

By expecting a response rate of 50% of cases that will reach the threshold of -1.05 D of the K_{max} value, a sample size of 42 patients achieves 91% power to detect a difference of 0,25 between the area under the ROC curve (AUC) under the null hypothesis of 0,60 and an AUC under the alternative hypothesis of 0,85 using a two-sided z-test at a significance level of 0,05000. The data are continuous responses. Considering a ≤20% drop-out rate (determined from systematic literature review on controlled and open clinical studies on standard and epithelium-on CXL procedures), a number of 50 participants (25 per treatment protocol) is allowed to be enrolled in the study.

Details on the methodology and process used to define the sample size of this clinical trial are described in Appendix 1 – Methodology for sample size calculation.

Statistical methodology

Descriptive statistics will be used to summarize the data: numerical variables will be summarized as mean and standard deviation or median and interquartile range, respectively, according to the distribution of the data; categorical variables will be represented as frequencies and proportions. Student's t-test or Wilcoxon test will be applied to compare continuous variables, while Fisher's exact test or Chi-Squared test will be used to analyze categorical variables. Bonferroni correction is applied to analysis of exploratory outcome measures of stratification groups. For all reported AEs, the data can be presented with events listed in order of decreasing frequency.

The optimal cut-off values in predicting the propensity of CXL treatment to flatten the Kmax value at 12-months was determined based upon the results of preclinical studies (data not disclosed to investigators to avoiding methodological study bias) and can be optimized with the Youden index.

The accuracy and precision (95% CI) of the combined use of the theranostic imaging biomarkers to predict CXL treatment outcome will be determined by calculating the proportion of correctly classified eyes and the positive predictive value (PPV) respectively. The miss rate, which is the probability that a true positive is missed by the theranostic methodology, will be determined by calculating the false negative rate (FNR).

Statistical significance will be set at 0.05. All the analysis will be performed using the statistical software R (latest version available).

Population to be analysed

The **Intent-to-Treat (ITT) Population** consists of all participants who were enrolled into the trial and performed at least 1 follow-up visit.

The **Per Protocol (PP) Population** will be a subset of the ITT population and will consists of all participants who participated and completed at least 66% of all the visits (i.e., 4 of 6 total visits) and the last visit 6 at day-360 and in whom there are no major protocol deviations.

Both populations will be agreed upon by Sponsor, Principal Investigator/Site of Investigation after the final subject visit and in advance of the database lock.

Ethical and Regulatory Issues

This study adheres with the ethical principles that have their origin in the Declaration of Helsinki (Seventh revision, 2013), the Convention of Oviedo, April, 4th 1997, and is consistent with GCP, including the ICH Guideline for good clinical practice E6, the Directive 2001/20/EC, the ISO 14155:2011, the Regulation (EU) 536/2014 of the European Parliament and of the Council of 16 April 2014, the Regulation (EU) 2017/745 of the European Parliament and of the Council of 5 April 2017, the Ministerial decree of the Italian Minister of Health of 2 August 2005, the Decree n.211 of 24 June 2003, the Ministerial Decree of 14 July 2009 and applicable regulatory requirements concerning human subject protection and other aspects of clinical investigations.

The study is conducted in strict compliance with the study protocol; any revision to study protocol must be discussed with and prepared by the Sponsor. The Investigator should not implement any deviation or change to the protocol without prior review and documented approval/favorable opinion from the Sponsor and IEC of an amendment. Any significant deviation must be documented in the CRF.

Good Clinical Practice (GCP) for Clinical trials

The Study will be conducted in accordance with the 13 principles of the rules of good clinical practice, as described below:

1) The study complies with the principles of the Declaration of Helsinki.

2) The expected benefits to participants justify the risks.

3) The rights, safety, and well-being of the participants to the trial are the most important considerations and prevail over those of science and society.

4) The available information about the investigational product are adequate to support the proposed clinical trial.

5) The clinical study is drafted according to strong technical and scientific background and is fully described in the study protocol.

6) The study will be conducted in accordance with the study protocol only after approval of the ethical committee.

7) The medical care given to, and the medical decisions made on behalf of the participants will always fall under the responsibility of qualified medical specialists.

8) All the investigators have the education, training, and experience to perform their specific tasks.

9) An informed consent should be freely obtained by each participant prior to his participation in the study.

10) Any information concerning the clinical study is recorded, handled, and stored in a way that allows its accurate reporting, interpretation and verification.
11) The confidentiality of the documents is ensured by respecting the privacy and confidentiality rules provided for by the applicable regulatory requirements.
12) The investigational products are manufactured, handled, and stored in compliance with good manufacturing practice applicable and are used according to the approved protocol.
13) Procedures are implemented to guarantee the quality of every aspect of the trial.

Independent Ethics Committee approval

The International Conference on Harmonisation (ICH) guidelines require that approval be obtained from an independent ethical committee (IEC) prior to participation of human participants in research studies. Prior to the study onset, the protocol, informed consent, advertisements to be used for participant recruitment, and any other written information regarding this study to be provided to the participant must be approved by the IEC. Documentation of all IEC approvals and of the IEC compliance with ICH Guideline E6 will be maintained by the site and will be available for review by the sponsor or its designee.
The Principal Investigator/Site of Investigation and CRO, if applicable, will support the Sponsor in answering question from the IEC in relation to the study or study procedures.
All IEC approvals should be signed by the IEC Chairman or designee and must identify the IEC name and address, the clinical protocol by title and/or protocol number and the date approval and/or favorable opinion was granted.
The study may only begin after approval of the same committee. Before initiating the clinical trial, the Principal Investigator/Site of Investigation should have written and dated approval/favorable opinion from the IEC for the trial protocol.

Informed consent

A written informed consent (Annex 3) shall be obtained from each participant prior to entering the study or performing any unusual or non-routine procedure that involves risk to the participant. An informed consent template may be provided by the sponsor to investigative sites. If any institution-specific modifications to study-related procedures are proposed or made by the site, the consent should be reviewed by the sponsor and/or its designee, if appropriate prior to IEC submission. Once reviewed, the consent will be submitted by the Principal Investigator/Site of Investigation to the IEC for review and approval prior to the start of the study. If the informed consent form is revised during the course of the study, all active participating participants must sign the revised form.

Before recruitment and enrollment, each prospective participant will be given a full explanation of the study and allowed to read the approved informed consent form. Once the Principal Investigator/Site of Investigation is assured that the participant understands the implications of participating in the study, the participant will be asked to give consent to participate in the study by signing the informed consent form.

The Principal Investigator/Site of Investigation shall provide an original form of the signed informed consent to the participant. A second original form shall be maintained in the participant's medical records at the site.

Risk Assessment and Management

The present study does not accept healthy volunteers. Participants who are eligible to the study are those affected by keratoconus.

This particular study design assumes that the theranostic-guided riboflavin/UV-A corneal cross-linking treatment does not induce in the participants unacceptable risks. Several clinical studies[10,11,12,13,14] have shown safety and efficacy of corneal cross-linking using a 0.2% riboflavin ophthalmic solution to protect the cornea prior to UV-A irradiation. The occurrence of severe adverse events, such as ulcerative keratitis, have been registered in 0.2% of Epi-OFF CXL procedures; no severe adverse events have been recorded after Epi-ON CXL procedures.

The safety of participants has been assessed according to the following issues:

1. The use of CE marked medical devices for the indication of use;
2. the risks inherent in the type and route of administration of the products;

The seriousness of possible adverse reactions and the probability of them happening have been considered during study design and listed under "Adverse Events" section. The innovative corneal cross-linking procedure is expected to elevate the gold standard practice by improving predictability of CXL clinical outcome and thus to provide adequate therapeutic benefit to justify entering keratoconus patients into the study. The management of the possible adverse reactions has been described under "Adverse Events" section.

The Sponsor has given the Principal Investigator/Site of Investigation and IEC full access to the relevant pre-clinical work on the medical devices in the Investigator's Brochure (Annex 8). The

[10] Zhang X, Sun L, Chen Y, Li M, Tian M, Zhou X. One-year outcomes of pachymetry and epithelium thicknesses after accelerated (45 mW/cm^2) transepithelial corneal collagen cross-linking for keratoconus patients. Sci Rep 2016;6:32692.

[11] Artola A, Piñero DP, Ruiz-Fortes P, Soto-Negro R, Pérez-Cambrodí RJ. Clinical outcomes at one year following keratoconus treatment with accelerated transepithelial cross-linking. Int J Ophthalmol 2017;10(4):652-655.

[12] Tian M, Jian W, Sun L, Shen Y, Zhang X, Zhou X. One-year follow-up of accelerated transepithelial corneal collagen cross-linking for progressive pediatric keratoconus. BMC Ophthalmol 2018;18(1):75.

[13] Kır MB, Türkyılmaz K, Öner V. Transepithelial high-intensity cross-linking for the treatment of progressive keratoconus: 2-year outcomes. Curr Eye Res 2017;42(1):28-31.

[14] Huang J, Shen Y, Jian W, Xu H, Li M, Zhao J, Zhou X, Liao H. Two-year topographic and densitometric outcomes of accelerated (45 mW/cm^2) transepithelial corneal cross-linking for keratoconus: a case-control study. BMC Ophthalmol 2018;18(1):337.

medical devices have been shown to be designed and manufactured according to the Directive 93/42/CE and to be further safe for the indication of use in pre-clinical and regulatory testing.
The Sponsor ensures for the entire duration of the study to monitor the safety of subjects by strict assessment of the risks inherent in the type of medical device and its target and in any potential immediate toxicity predicted from non-clinical or literature information.

Sites of Investigation

- Azienda Ospedaliero Universitaria Careggi – Università di Firenze

The Careggi University Hospital has a public legal personality and is empowered with entrepreneurial, organizational, administrative, patrimonial, financial, managerial and technical autonomy. It is a Trust integrated with the University of Florence and is characterized by the health care activities of hospitalization, specialized outpatient services and Emergency Room/Urgent care services. It pursues the development of high standard specialization and is a Reference in the Florentine Wider Area as well as on a regional and national scale. The Corneal Unit of the Ophthalmology department is the clinical unit involved in the present clinical study. The Unit is engaged in the validation of new methods of diagnosis and treatment of corneal and anterior segment eye diseases under the responsibility of Dr. Rita Mencucci. Both the research activities and the clinical and surgical activities are carried out at the at main Careggi University Hospital site.

- Azienda Ospedaliera Universitaria Policlinico G. Martino – Università di Messina

The G. Martino University hospital has a public legal personality and is a Trust integrated with the University of Messina. It is characterized by the health care activities of hospitalization, specialized outpatient services and Emergency Room/Urgent care services. It pursues the development of high standard specialization and is a Reference in the regional scale. The Corneal Unit of the Ophthalmology department is the clinical unit involved in the present clinical study. This Unit is engaged in the validation of new methods of diagnosis and treatment of ocular surface, corneal and anterior segment eye diseases under the responsibility of Dr. Anna Maria Roszkowska. Both the research activities and the clinical and surgical activities are carried out at the the Policlinico G. Martino.

- Azienda Ospedaliera Universitaria Mater Domini – Università Magna Graecia di Catanzaro

The Mater Domini University hospital has a public legal personality and is a Trust integrated with the University of Catanzaro. It is a modern structure pursuing the development of high standard specialization and is a Reference in the regional scale. The Ophthalmology department is the clinical unit involved in the present clinical study. The department is engaged in the validation of new methods of diagnosis and treatment of eye diseases under the responsibility of Prof. Vincenzo Scorcia.

Investigator qualifications

- Prof. Rita Mencucci graduated in Medicine and Surgery at the University of Florence in 1984 with 100/100 and honors. She specialized in Ophthalmology at the University of Florence in 1988 with full marks and honors. She is Professor at the school of ophthalmology of the University of Florence. Member of numerous scientific societies. Author of numerous publications in peer-reviewed scientific journals, she has also been a speaker at numerous national and international conferences. Her main area of expertise includes keratoconus management and corneal cross-linking.
- Prof. Anna Maria Roszkowska graduated in Medicine and Surgery in 1987 at the University Jagiellonia of Cracovia (Poland) with 110/110 *cum laude*. She holds a PhD in Refractive Surgery at the University of Padua in 1999. She is associate professor of Ophthalmology at the University of Messina. Author of numerous publications in peer-reviewed scientific journals, she has also been a speaker at numerous national and international conferences. Her main area of expertise includes keratoconus management and corneal cross-linking.
- Prof. Vincenzo Scorcia graduated in Medicine and Surgery at the University of Rome “La Sapienza” in 2001 with 110/110 and honors. He specialized in Ophthalmology at the University of Magna Græcia of Catanzaro in 2006 with 90/90 and honors. He is author of numerous publications in peer-reviewed scientific journals and a speaker at numerous national and international conferences. He has been researcher at the Faculty of Medicine and Surgery of the University of Magna Græcia of Catanzaro since 2008. He has been Associate Professor, Director of the Ophthalmology Unit and the Specialization School in Ophthalmology since 2016.

Sponsor

Regensight s.r.l. (hereinafter “Regensight” or the “startup company”) was established in 26 July 2019 in order to develop and introduce the theranostics technology in eye care.

The company’s legal form is a limited liability company. It is registered with n. RM-1586517 in the special section for innovative startup Co. in the Chamber of Commerce of Roma, VAT n. IT15376761001.

The legal representative of Regensight is Dr. Marco Lombardo. Dr. Marco Lombardo was graduated with honours in Medicine and Surgery and specialized with honours in Ophthalmology. He holds a PhD in Biomedical Engineering and Computer Science. He has developed several innovative medical devices for the diagnosis and treatment of eye diseases, including adaptive optics retinal imaging, corneal iontophoresis for treating keratoconus, scleral iontophoresis for the prevention of AMD progression, femtosecond laser for cataract surgery. He has been working as senior researcher at an ophthalmic IRCCS in Rome for 9 years. He has been one of expert members of the Technical Scientific Direction of the Italian Ministry of Health, member of the expert committee for the clinical application of new technologies in Visual Science of OSA, member of the Association of Research in Vision and Ophthalmology (ARVO), the European Society of Cataract and Refractive Surgery (ESCRS) and the Società Italiana di Oftalmologia (SOI). He published more than 100 papers in peer-reviewed scientific journals. He is inventor of 3 patent families.

Administrative Rules

Clinical data handling

The Sponsor is responsible for communicating the start, end and eventual discontinuation of the trial to the Regulatory Authorities and Independent Ethical Committees (IEC).

The Clinical Investigators are required to ensure that the data collected during the study protocol are accurate, complete, verifiable and traceable. In addition, they are obliged to inform the IEC and the Sponsor of all serious and unexpected adverse events related to medical devices used in the study, in accordance with the Legislative Decree n. 211/2003.

The Clinical Investigators are required to send to the Sponsor a detailed final report on the outcome thereof. The Sponsor and Principal Investigator are required to write a final report on the study outcome, which will be forwarded to the IECs.

The Sponsor is required to notify the IECs about any changes to the present study protocol.

Before commencing the present study, the Sponsor and the Sites of Investigation will sign a Clinical Trial Agreement (Annex 10). The identification codes and the CRF of each participant recruited in the present study will be archived by the Sponsor and the Site of Investigation until the time limit set by law.

Case Report Forms

As part of the responsibilities assumed by participating in the study, the clinical investigators agree to maintain adequate case histories for the participants treated as part of the research under this protocol. The clinical investigators agree to maintain accurate CRFs (Annex 1) and source documentation as part of the case histories according to the instructions to complete CRF (Annex 2). The Sponsor will supply the CRFs. All CRFs should be completed legibly in black ink. The CRFs may not be completed in pencil.

All CRF information is to be filled in. If an item is not available or is not applicable, this fact should be indicated. Blank spaces should not be present unless otherwise directed. A correction should be made by striking through the incorrect entry with a single line and the corrected information should be entered adjacent to the deleted item. The correction must be initialed and dated by the person making the correction.

Each completed CRF must be reviewed, signed, and dated by the principal investigator and clinical monitor in a timely manner. The completed CRF will be checked by study monitors as soon as practical after completion. Scan copies of the CRF pages required to elaborate the interim report will be sent to the statistician responsible for the analysis of data during the study. At the end of the study

the site will send a scanned copy of all CRF to the Sponsor and will retain the originals at the site in the principal investigator's files.

Reports

Each site of Investigation will provide information about study progression to the Sponsor on a three months basis after the enrolment of first patient into the study. This information will be reported by the Sponsor in the form (Annex 5) provided by the Sponsor to the Principal Investigator/Site of Investigation.

In addition, each site of Investigation shall write, in English, an interim report (Annex 6) including all patients who have completed visit 5 (day 180), according to the ICH E3 guidance on structure and content of clinical study reports.

Each form must be filled with the following information:

- number of subjects visited at baseline (and/or reason for enrolment failure) (visit 1);
- number of subjects recruited (visit 1);
- number of subjects receiving the treatment (day 0);
- Average intracorneal concentration of riboflavin after dosing (day 0).
- Average intracorneal concentration of riboflavin at the end of treatment (day 0).
- Average Treatment Efficacy Index (day 0).
- Number of subjects completing visits after treatment.
- number of premature terminations/drop-outs.
- Average UDVA at each visit.
- Average CDVA at each visit.
- Average manifest refraction at each visit.
- Average ECD at each visit.
- Slit lamp biomicroscopy data at each visit.
- Interim analysis of primary outcome measure at 6-months.
- Adverse events (number and type).
- Missing data.
- Other information (if any).

A final report (Annex 7) shall be elaborated in written (English) according the ICH E3 guidance on structure and content of clinical study reports and sent to the Sponsor for approval. The Final Report will compile all the data collected from all the participants during the entire duration of the study (from screening to the end of follow-up, including AE (if any), discussion of statistical analysis as well as interpretation of the results. This report will be sent to the Sponsor within 2 weeks from the final

monitoring visit performed by the Sponsor to confirm the correctness and completeness of the data collected and database lock. In the Final Report, a clear conclusion on the following points must be provided:

- Assessment of primary outcome measure at 12 months (day 360), such as:
    - Validation of the combined use of the theranostic imaging biomarkers. True positives, false positives, true negatives and false negatives were evaluated in order to determine the ability of the theranostic imaging biomarkers, including both the *riboflavin score* and *theranostic score*, to predict the propensity of CXL treatment in flattening the K_{max} value (D) by more than 0.1 D at 12 months postoperatively.
- Assessment of all secondary outcome measures, such as:
    - Change of Maximum Keratometry assessed by corneal topography at 12-months.
    - Change of Endothelial Cell Density assessed by specular microscopy at 12-months.
- Assessment of the additional exploratory outcome measures, such as:
    - Change of Corrected Distance Visual Acuity (CDVA) assessed by ETDRS at 12-months.
    - Change of Uncorrected Distance Visual Acuity (UDVA) assessed by ETDRS at 12-months.
    - Change of Manifest Refraction assessed by ETDRS at 12-months.
    - Change of secondary outcome measures at 12-months in either stratification group.

Study Reporting Requirements

By participating in this study, the Principal Investigator/Site of Investigation agree to provide the Sponsor with the adequate study reports, as defined in the “Reports” section above.
The Principal Investigator/Site of Investigation agree to submit reports of AEs and SAEs according to the timeline and method outlined in the protocol. In addition, the Principal Investigator/Site of Investigation agree to submit a report to the IEC as appropriate.

Monitoring of the Study

The Site of Investigation has the obligation to follow the study closely and to support the by Sponsor in monitoring the study. In doing so, the Principal Investigator/Site of Investigation will assist the Sponsor to visit the study facility as defined in the monitoring plan, in addition to maintaining necessary contact through telephone, email, and letter. The Sponsor will maintain current personal knowledge of the study through observation, review of study records and source documentation, and discussion of the conduct of the study with the principal investigator or staff. All aspects of the study

must be carefully monitored for compliance with applicable government regulation with respect to current GCP and current standard operating procedures.

Monitoring Plan

On-site monitoring will be performed by the Sponsor at the Site of Investigation before commencing the study and at the end of the study (after visit 6, i.e., 360 days after all participants have been treated). On-site monitoring will be carried out to verify that:

- the Principal Investigator receives the current Investigator's Brochure, all documents, and all trial supplies needed to conduct the trial properly and to comply with the applicable regulatory requirement(s).
- the Principal Investigator and Study Staff have adequate experience and have been adequately informed and trained to carry the study.
- the Site of Investigation has adequate resources to carry the study.
- the investigators follows the approved protocol and all approved amendment(s).
- written informed consent was obtained before each subject's participation in the trial.
- the storage of the RitSight™ are acceptable, and that supplies are sufficient throughout the trial.
- the disposition of unused investigational product(s) at the trial sites complies with applicable regulatory requirement(s) and is in accordance with the sponsor.
- the RitSight™ is supplied only to subjects who are eligible to receive it and at the protocol specified doses.
- the CRF entries and other operating documents are filled with accuracy and completeness by Investigators.
- If CRFs have any entry error, omission, or illegibility, informing the investigator of it. The Sponsor shall ensure that appropriate corrections, additions, or deletions are made, dated, explained (if necessary), and initialed by the principal investigator.
- all adverse events are appropriately reported within the time periods required by GCP, the protocol, the IEC, the Sponsor, and the applicable regulatory requirement(s).
- if deviations from the protocol occur, communicating the applicable regulatory requirements to the investigator and taking appropriate action designed to prevent recurrence of the detected deviations.

Centralized monitoring will be performed by the Sponsor to review interim and final reports provided by the Principal Investigation/Site of Investigation. Review will include analyses of study data, including the following actions:

- to identify missing data, inconsistent data, data outliers, unexpected lack of variability and protocol deviations.
- to evaluate for systematic or significant errors in data collection and reporting at a site or across sites; or potential data manipulation or data integrity problems.
- to perform statistical analysis of study data.

Inspection of Records

The Principal Investigator/Site of Investigation will permit, at any working day, trial-related monitoring, audits, IEC review, and regulatory inspection(s) by providing direct access to all study records. In the event of an audit, the Principal Investigator/Site of Investigation agrees to allow the Sponsor, representatives of the sponsor, the Regulatory authorities to access to all study records.
The Principal Investigator/Site of Investigation should promptly notify the Sponsor of any audits scheduled by any regulatory authorities and promptly forward copies of any audit reports received to the sponsor.

Study Record Retention

Essential documents should be retained until at least 5 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or at least 3 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents should be retained for a longer period, however, if required by the applicable regulatory requirements or by an agreement with the Sponsor. It is the responsibility of the Sponsor to inform the Principal Investigator/Site of Investigation as to when these documents no longer need to be retained.

Quality Assurance

The Sponsor is responsible for implementing and maintaining quality assurance and quality control systems to ensure that the study is conducted and data are generated, documented and reported in compliance with the protocol, GCP and the applicable regulatory requirements. The Sponsor ensures that case report forms, and other operational documents are clear, concise, and consistent.
The Sponsor shall make sure that each C4V CHROMO4VIS™ system and each batch of the RitSight™ eye drop meets the requirements and that is properly delivered to the Site of Investigation.
The Principal Investigator/Site of Investigation ensures the accuracy, completeness, legibility, and timeliness of the data reported to the Sponsor in the CRFs and in all required reports. The Principal Investigator/Site of Investigation ensures that there is adequate training for all staff participating in

the conduct of the study, and that they are aware of regulatory requirements and acceptable standards for the conduct of clinical trials and the protection of human subjects.
The Sponsor ensures to monitor that all operating documents are using standard terminology and to provide systematic safeguard to ensure adherence to study protocol by the Principal Investigator/Site of Investigation.

Confidentiality

All evaluation forms, reports, and other records will be identified in a manner designed to maintain participant confidentiality. All records will be kept in a secure storage area with limited access. Clinical information will not be released without the written permission of the participant [or the participant's legal guardian], except as necessary for monitoring and auditing by the sponsor, its designee, the regulatory authorities, or the IEC.
The Principal Investigator/Site of Investigation shall not disclose or use for any purpose other than performance of the study, any data, record, or other unpublished, confidential information disclosed to those individuals for the purpose of the study. Prior written agreement from the Sponsor must be obtained for the disclosure of any said confidential information to other parties.

Personal data protection

The study adheres to the General Data Protection Regulation (GDPR). The rights of each participant to the study to physical and mental integrity, to privacy and to the protection of the data ("personal data") concerning him/her in accordance with data protection laws, are safeguarded. Personal data include any information relating to an identified or identifiable natural person ('data subject'); an identifiable person is one who can be identified, directly or indirectly, in particular by reference to an identification number or to one or more factors specific to his physical, physiological, mental, economic, cultural or social identity. The Sponsor monitors whether the Principal Investigator/Site of Investigation performs the study in conformity with the protocol and safeguard personal data. In this study, data records are key-coded data and thus any other person cannot de-code the data easily. The Sponsor and the Principal Investigator/Site of Investigation will take appropriate actions to safeguard personal data of the study. The data controller is the legal representative of the Sponsor; the data processor is the Principal Investigator.

Modification of the Protocol

Any changes in this research activity, except those necessary to remove an apparent, immediate hazard to the participant, must be reviewed and approved by the Sponsor. Amendments to the

protocol must be submitted in writing to the IEC for approval prior to participants being enrolled into an amended protocol.

Protocol Violations and Deviations

The Principal Investigator/Site of Investigation must document and explain in the participant's source documentation any deviation from the approved protocol. The Principal Investigator/Site of Investigation may implement a deviation from, or a change of, the protocol to eliminate an immediate hazard to trial participants without prior IEC approval. As soon as possible after such an occurrence, the implemented deviation or change, the reasons for it, and any proposed protocol amendment(s) should be submitted to the IEC for review and approval, to the sponsor for agreement, and to the regulatory authorities, if required.

A deviation from the protocol is an unintended and/or unanticipated departure from the procedures and/or processes approved by the Sponsor and the IEC and agreed to by the Principal Investigator/Site of Investigation. Deviations usually have an impact on individual participants or a small group of participants and do not involve inclusion/exclusion or primary endpoint criteria. A protocol violation occurs when there is nonadherence to the protocol that results in a significant, additional risk to the participant, when the participant or Principal Investigator/Site of Investigation has failed to adhere to significant protocol requirements (inclusion/exclusion criteria) and the participant was enrolled without prior sponsor approval, or when there is nonadherence to authorities regulations.

The Sponsor will document protocol violations and deviations throughout the course of monitoring visits. The Sponsor will notify the principal investigator during a visit and/or in writing of all violations and deviations. The IEC should be notified of all protocol violations and deviations in a timely manner.

List of major deviations/violation

- Patient not met inclusion criteria
- Patient not met exclusion criteria
- Not obtained signed consent form from patient
- Corneal topography not done at visit 1 (baseline)
- Corneal topography not done at visit 6 (day 360)
- Corneal cross-linking treatment not completed

Financial Disclosure and Obligations

The clinical investigators are required to provide financial disclosure information to allow the Sponsor to submit the complete and accurate certification or disclosure statements to regulatory authorities. In addition, the principal investigator or investigators must provide to the Sponsor a commitment to promptly update this information if any relevant changes occur during the course of the investigation. The clinical investigators are required to provide an updated curriculum vitae (CV).

The Sponsor is not financially responsible for further testing/treatment of any medical condition, which may be detected during the screening process. In addition, in the absence of specific arrangements, the Sponsor is not financially responsible for further treatment of the participant's disease.

Study Conduct

The Principal Investigator/Site of Investigation agree that the study will be conducted according to the principles of the ICH E6 Guideline for Good Clinical Practice and the principles of the World Medical Association Declaration of Helsinki. The Principal Investigator/Site of Investigation will conduct all aspects of this study in accordance with all national, state, and local laws or regulations.

Recruitment strategies

The screening and the recruitment processes will respect the dignity and autonomy of the potential participants by avoiding any potential undue influence and by protecting both the privacy of the individual and the confidentiality of any information obtained for recruitment and/or screening purposes.

In preparing recruitment materials the Principal Investigator/Site of Investigation shall consider the purpose of the research and the setting in which the research will be conducted. The following methods of recruiting subjects will be considered acceptable:

- <u>Direct recruitment of potential study participants</u>

Examples of this strategy are physicians talking with their own or clinic patients about the study, contact between the study team and potential subjects in person, on the phone or on the internet. With this method considerable care will have to be taken so that the person contacted does not feel pressured to participate.

- <u>Referrals</u>

Referrals may be from non-investigator healthcare providers, participants referring other participants.

- Advertisements, flyers, information sheets, notices, internet postings and/or media can be used to recruit subjects.

Prospective participants who respond to these will contact directly the Site of Investigation.
Recruitment materials can contain the following Information:

1. The name of the institution(s);
2. The name of the Principal Investigator(s) and Sponsor, and the name of a contact person with a telephone number and email address to call and write for information about the study;
3. The purpose of the research and, in summary form, the eligibility criteria that will be used to admit subjects into the study;
4. The location of the research and time commitment, if appropriate.

The Promoter and Sponsor can prepare advertisements, information sheets, notices, internet postings and/or media but cannot directly contact prospective subjects (see Annex 12). Per-participant incentive payments or referral fees, whether paid for each referral or each enrollment, are not allowed.

Publications

Clinical data are the property of the Sponsor and cannot be published without prior authorization from the Sponsor, but data and publication thereof will not be unduly withheld.
The data of the study may be considered for reporting at a scientific meeting or for publication(s) in a scientific journal. In these cases, the Sponsor will be responsible for these activities and will decide who will eventually present study outcomes at a scientific meeting, if and how the manuscript(s) is written and edited, the number and order of authors, the scientific journal(s) to which it/they will be submitted, and other related issues. The Sponsor has final approval authority over all such issues.

Study Termination

The Sponsor may prematurely terminate the study in case of major violations, which may prevent the safety of the subjects or future evaluation of the study results. In addition, the Sponsor, may prematurely terminate the study, in its sole discretion and for any reason whatsoever associated with changes in its commercial or clinical strategy.
In such an occurrence the Sponsor shall give at least thirty (30) calendar days' written notice of termination to the study centre. All the effort will be made to perform a final visit to all the subjects still in the study aiming to confirm their wellbeing.

References

(in alphabetical order)

1. Akpek EK, Dart JK, Watson S, Christen W, Dursun D, Yoo S, et al. A randomized trial of topical cyclosporine 0.05% in topical steroid-resistant atopic keratoconjunctivitis. Ophthalmology 2004; 111: 476-482.
2. Alio JL, Abbouda A, Valle DD, Del Castillo JM, Fernandez JA. Corneal cross-linking and infectious keratitis: a systematic review with a meta-analysis of reported cases. J Ophthalmic Inflamm Infect 2013;3(1):47.
3. Angunawela RI, Arnalich-Montiel F, Allan BD. Peripheral sterile corneal infiltrates and melting after collagen crosslinking for keratoconus. J Cataract Refract Surg 2009; 35(3): 606-607.
4. Baenninger PB, Bachmann LM, Wienecke L, Kaufmann C, Thiel MA. Effects and adverse events after CXL for keratoconus are independent of age: a 1-year follow-up study. Eye (Lond) 2014; 28(6): 691-695.
5. Beshtawi IM, O'Donnell C, Radhakrishnan H. Biomechanical properties of corneal tissue after ultraviolet-A-riboflavin crosslinking. J Cataract Refract Surg 2013; 39(3): 451-462.
6. Chan E, Snibson GR. Current status of corneal collagen cross-linking for keratoconus: a review. Clin Exp Optom 2013; 96(2): 155-164.
7. Cheung I, McGhee C, Sherwin T. A new perspective on the pathobiology of keratoconus: interplay of stromal wound healing and reactive species-associated processes. Clin Exp Optom 2013; 96: 188-196.
8. Chan E, Snibson GR. Current status of corneal collagen cross-linking for keratoconus: a review. Clin Exp Optom 2013; 96(2): 155-164.
9. Chunyu T, Xiujun P, Zhengjun F, Xia Z, Feihu Z. Corneal collagen cross-linking in keratoconus: a systematic review and meta-analysis. Sci Rep 2014; 4: 5652.
10. Dhawan S, Rao K, Natrajan S. Complications of corneal collagen cross-linking. J Ophthalmol 2011; 2011: 869015.
11. Doughty MJ, Cullen AP. Long-term effects of a single dose of ultraviolet-B on albino rabbit cornea. In vivo analyses. Photochem Photobiol 1989; 49:185-196.
12. Fantes FE, Hanna KD, Waring III GO, Pouliquen Y, Thompson KP, Savoldelli M. Wound healing after excimer laser keratomileusis (photorefractive keratectomy) in monkeys. Arch Ophthalmol 1990; 108: 665-675.
13. Ferdi A, et al. Keratoconus Natural Progression: A Systematic Review and Meta-analysis of 11 529 Eyes. Ophthalmology 2019;126:935-945.

14. Huang AJW, Tseng SCG, Kenyont KR. Paracellular permeability of corneal and conjunctival epithelia. Invest Ophthalmol Vis Sci 1989; 30(4): 684-689.
15. Godefrooij DA, Roohé SL, Soeters N, Wisse RPL. The Independent Effect of Various Cross-Linking Treatment Modalities on Treatment Effectiveness in Keratoconus. Cornea 2020;39:63–70.
16. Gordon-Shaag A, Millodot M, Shneor E, Liu Y. The Genetic and Environmental Factors for Keratoconus. Biomed Res Int 2015; 2015: 795738.
17. Greenstein SA, Fry KL, Bhatt J, Hersh PS. Natural history of corneal haze after collagen crosslinking for keratoconus and corneal ectasia: Scheimpflug and biomicroscopic analysis. J Cataract Refract Surg 2010; 36: 2105-2114.
18. Kinsey VE. Spectral transmission of the eye to ultraviolet radiations. Arch Ophthalmol 1948; 39:508-513.
19. Koller T, Mrochen M, Seiler T. Complication and failure rates after corneal crosslinking. J Cataract Refract Surg 2009; 35: 1358-1362.
20. Kolozsvari L, Nogradi A, Hopp B, Bor Z. UV Absorbance of the human cornea in the 240- to 400-nm Range. Invest Ophthalmol Vis Sci 2002; 43:2165–2168.
21. Lembares A, Hu X-H, Kalmus GW. Absorption spectra of corneas in the far ultraviolet region. Invest Ophthalmol Vis Sci 1997; 38:1283–1287
22. Li J Ji P, Lin X. Efficacy of corneal collagen cross-linking for treatment of keratoconus: a meta-analysis of randomized controlled trials. PLoS One. 2015; 10(5): e0127079.
23. Lombardo M, Pucci G, Barberi R, Lombardo G. Interaction of ultraviolet light with the cornea: clinical implications for corneal crosslinking. J Cataract Refract Surg 2015; 41(2):446-459.
24. Lombardo G et al. Non-invasive optical method for real-time assessment of intracorneal riboflavin concentration and efficacy of corneal cross-linking. J Biophotonics 2018; Jul;11(7):e201800028.
25. Lombardo M, Lombardo G. Non-invasive and real time assessment of riboflavin consumption in standard and accelerated corneal cross-linking. J Cataract Refract Surg 2019; 45(1):80-86.
26. Lombardo G, Serrao S, Lombardo M. Comparison between standard and transepithelial corneal cross-linking using a theranostic UV-A device. Graefe's Arch Clin Exp Ophthalmol 2020; 5 Gen E-pub.
27. Lombardo M, Serrao S, Raffa P, Rosati M, Lombardo G. Novel technique of transepithelial corneal cross-linking using iontophoresis in progressive keratoconus. J Ophthalmol 2016; 7472542.

28. Lombardo M, Giannini D, Lombardo G, Serrao S. Randomized controlled trial comparing transepithelial corneal cross-linking using iontophoresis with the Dresden protocol in progressive keratoconus. Ophthalmology 2017; 124: 804-812.
29. Lombardo M, Serrao S, Lombardo G. Schiano-Lomoriello D. Two years outcomes of a randomized controlled trial of transepithelial corneal cross-linking with iontophoresis for keratoconus. J Cataract Refract Surg 2019 Apr 16. pii: S0886-3350(19)30060-4.
30. Lombardo M, Serrao S. Corneal Cross-Linking Standardized Terminology. J Refract Surg 2018; 34(3):213.
31. Meek KM, Hayes S. Corneal cross-linking–a review. Ophthal Physiol Opt 2013; 33:78–93
32. Oster G, Bellin JS, Holstrom B. Photochemistry of riboflavin. Experientia Vol XVIII – 1962; 6:249–296
33. Podskochy A. Protective role of corneal epithelium against ultraviolet radiation damage. Acta Ophthalmol Scand 2004; 82:714–717
34. Raiskup F, Hoyer A, Spoerl E. Permanent corneal haze after riboflavin-UVA-induced cross-linking in keratoconus. J Refract Surg 2009; 25: S824-S828.
35. Raiskup F, Theuring A, Pillunat LE, Spoerl E. Corneal collagen crosslinking with riboflavin and ultraviolet-A light in progressive keratoconus: Ten-year results. Cataract Refract Surg 2015; 41:41-46.
36. Raiskup F, Spoerl E. Corneal cross-linking with riboflavin and ultraviolet A. Part II. Clinical indications and results. Ocular Surf 2013; 11:93-108.
37. Randleman JB, Khandelwal SS, Hafezi F. Corneal Cross-Linking. Surv Ophthalmol 2015; 60(6):509-523.
38. Ringvold A. Corneal epithelium and UV-protection of the eye. Acta Ophthalmol Scand 1998: 76:149-153
39. Ringvold A. In vitro evidence for UV-protection of the eye by the corneal epithelium mediated by the cytoplasmic protein, RNA and ascorbate. Acta Ophthalmol Scand 1997; 75: 496–498
40. Shalchi Z, Wang X, Nanavaty MA. Safety and efficacy of epithelium removal and transepithelial corneal collagen crosslinking for keratoconus. Eye (Lond) 2015; 29(1): 15-29.
41. Soeters N, van der Valk R, Tahzib NG. Corneal cross-linking for treatment of progressive keratoconus in various age groups. J Refract Surg 2014; 30(7): 454-460.
42. Spoerl E, Raiskup F, Kampik D, Geerling G. Correlation between UV absorption riboflavin concentration in different depths of the corneal and CXL. Curr Eye Res 2010; 35:1040–1041
43. Sykakis E, Karim R, Evans JR, Bunce C, Amissah-Arthur KN, Patwary S, McDonnell PJ, Hamada S. Corneal collagen cross-linking for treating keratoconus. Cochrane Database Syst Rev 2015 Mar 24; 3: CD010621.

44. Tao X, Yu H, Zhang Y, Li Z, Jhanji V, Ni S, Wang Y, Mu G. Role of corneal epithelium in riboflavin/ultraviolet-A mediated corneal cross-linking treatment in rabbit eyes. J Ophthalmol 2013; 624563.
45. Taylor HR, West SK, Rosenthal FS, Munoz B, Newland HS, Emmett EA. Corneal changes associated with chronic UV irritation. Arch Ophthalmol 1989: 107:1481-1484.
46. Van den Berg TJ, Tan KE. Light transmittance of the human cornea from 320 to 700 nm for different ages. Vis Res 1994; 34:1453-156.
47. Zhang X, Zhao J, Li M, Tian M, Shen Y, Zhou X. Conventional and transepithelial corneal cross-linking for patients with keratoconus. PLoS One 2018; 13(4): e0195105.

Appendix 1 - Methodology for sample size calculation

Sample size calculation has been based on a thorough literature search on clinical investigations of the state of the art on corneal cross-linking for the treatment of keratoconus.

The inclusion criteria included:

A. Population: patients affected by keratoconus
B. Intervention: corneal cross-linking

Outcome/endpoint: change of maximum keratometry (K_{max}) measured with corneal topography.

The exclusion criteria included:

C. lack to report adequate information on study methodology (population, intervention, statistics);
D. follow-up shorter than 1-year follow-up after corneal cross-linking. This approach guarantees to analyze pertinent data with enough follow-up time to assess safety and performance of the UV-A medical device for the indication of use.
E. present case report or small (<15 participants) case series or have been published as a letter to the Editor;
F. are retrospective clinical studies;
G. have not been published or have been published in scientific journals without impact factor or in journal with impact factor lower than 1.5 (i.e., the last quartile of the JcR category “Ophthalmology” in 2018) This approach guarantees that proper scientific methodology has been used in order to drive significant conclusions on the results;
H. have been published earlier than 2013.

The search output provided the following scientific articles:

1. Chunyu T, Xiujun P, Zhengjun F, Xia Z, Feihu Z. Corneal collagen cross-linking in keratoconus: a systematic review and meta-analysis. Sci Rep 2014; 4: 5652.
2. Craig JA, Mahon J, Yellowlees A, Barata T, Glanville J, Arber M, Mandava L, Powell J, Figueiredo F. Epithelium-off photochemical corneal collagen cross-linkage using riboflavin and ultraviolet a for keratoconus and keratectasia: a systematic review and meta-analysis. Ocul Surf 2014;12(3):202-214.
3. Hersh PS, Stulting RD, Muller D, Durrie DS, Rajpal RK, United States Crosslinking Study Group. United States multicenter clinical trial of corneal collagen crosslinking for keratoconus treatment. Ophthalmology 2017; 124(9): 1259-1270.
4. Hersh PS, Stulting RD, Muller D, Durrie DS, Rajpal RK, U.S. Crosslinking Study Group. U.S. Multicenter clinical trial of corneal collagen crosslinking for treatment of corneal ectasia after refractive surgery. Ophthalmology 2017; 124(10): 1475-1484.

5. Jiang LZ, Jiang W, Qiu SY. Conventional vs. pulsed-light accelerated corneal collagen cross-linking for the treatment of progressive keratoconus: 12-month results from a prospective study. Exp Ther Med. 2017 Nov;14(5):4238-4244. doi: 10.3892/etm.2017.5031. Epub 2017 Aug
6. Kır MB, Türkyılmaz K, Öner V. Transepithelial High-Intensity Cross-Linking for the Treatment of Progressive Keratoconus: 2-year Outcomes. Curr Eye Res 2017;42(1):28-31.
7. Kobashi H, Rong SS. Corneal Collagen Cross-Linking for Keratoconus: Systematic Review. Biomed Res Int. 2017;2017:8145651
8. Kobashi H, Tsubota K. Accelerated versus standard corneal cross-linking for progressive keratoconus: a meta-analysis of randomized controlled trials. Cornea 2020; 39(2): 172-180.
9. Li J, Ji P, Lin X. Efficacy of corneal collagen cross-linking for treatment of keratoconus: a meta-analysis of randomized controlled trials. PLoS One. 2015 May 18;10(5):e0127079.
10. Mazzotta C, Traversi C, Paradiso AL, Latronico ME, Rechichi M. Pulsed Light Accelerated Crosslinking versus Continuous Light Accelerated Crosslinking: One-Year Results. J Ophthalmol 2014; 2014: 604731.
11. McAnena L, Doyle F, O'Keefe M. Cross-linking in children with keratoconus: a systematic review and meta-analysis. Acta Ophthalmol 2017;95(3):229-239.
12. Meiri Z, Keren S, Rosenblatt A, Sarig T, Shenhav L, Varssano D. Efficacy of Corneal Collagen Cross-Linking for the Treatment of Keratoconus: A Systematic Review and Meta-Analysis. Cornea 2016;35(3):417-428.
13. Shajari M, Kolb CM, Agha B, Steinwender G, Müller M, Herrmann E, Schmack I, Mayer WJ, Kohnen T. Comparison of standard and accelerated corneal cross-linking for the treatment of keratoconus: a meta-analysis. Acta Ophthalmol 2019;97(1):e22-e35.
14. Tomita M, Mita M, Huseynova T. Accelerated versus conventional corneal collagen crosslinking. J Cataract Refract Surg 2014;40(6):1013-1020.
15. Wen D et al. Comparison of epithelium-Off versus transepithelial corneal collagen cross-Linking for keratoconus: a systematic review and meta-analysis. Cornea 2018;37(8):1018-1024.
16. Wen D, Li Q, Song B, Tu R, Wang Q, O'Brart DPS, McAlinden C, Huang J. Comparison of Standard Versus Accelerated Corneal Collagen Cross-Linking for Keratoconus: A Meta-Analysis. Invest Ophthalmol Vis Sci 2018;59(10):3920-3931.
17. Zhang X, Zhao J, Li M, Tian M, Shen Y, Zhou X. Conventional and transepithelial corneal cross-linking for patients with keratoconus. PLoS One 2018; 13(4): e0195105.

The analysis of pertinent data from **6.995 cases** treated by corneal cross-linking has shown an average K_{max} change of **-1.05±0.80 D after 1 year follow-up**, showing that the treatment is effective for stabilizing the cornea, which has been weakened by disease or laser surgery.
The average K_{max} change was **-0.79±0.75 D at 6-month follow-up**.

A further search was done to understand whether the CXL treatment protocol (i.e., standard CXL or transepithelial CXL) may influence the change of K_{max} at 1-year follow-up.
The inclusion criteria included:

I. Population: patients affected by keratoconus
J. Intervention: corneal cross-linking
K. Type of study: randomized controlled trial

Outcome/endpoint: change of maximum keratometry (K_{max}) measured with corneal topography.
The exclusion criteria included:

L. lack to report adequate information on study methodology (population, intervention, statistics);
M. have follow-up shorter than 1-year follow-up after corneal cross-linking. This approach guarantees to analyze pertinent data with enough follow-up time to assess safety and performance of the UV-A medical device for the indication of use.
N. present case report or small (<15 participants) case series or have been published as a letter to the Editor;
O. are retrospective clinical studies;
P. are meta-analysis studies;
Q. have not been published or have been published in scientific journals without impact factor or in journal with impact factor lower than 1.5 (i.e., the last quartile of the JcR category “Ophthalmology” in 2018) This approach guarantees that proper scientific methodology has been used in order to drive significant conclusions on the results.
R. Have been published earlier than 2013.

The search output provided the following scientific articles:

1. Hersh PS et al. United States Crosslinking Study Group. United States multicenter clinical trial of corneal collagen crosslinking for keratoconus treatment. Ophthalmology 2017; 124(9): 1259-1270.
2. Wittig-Silva et al. A Randomized, controlled trial of corneal collagen cross-linking in progressive keratoconus. Three-Year Results. Ophthalmology 2014; 121:812-821.

3. Soeters N et al. Transepithelial versus epithelium-off corneal cross-linking for the treatment of progressive keratoconus: a randomized controlled trial. Am J Ophthalmol 2015;159(5):821-8.e3.
4. Hashemian H et al. Evaluation of corneal changes after conventional versus accelerated corneal cross-linking: a randomized controlled trial. J Refract Surg 2014;30(12):837-42.
5. Lombardo M et al. Randomized controlled trial comparing transepithelial corneal cross-linking using iontophoresis with the Dresden protocol in progressive keratoconus. Ophthalmology 2017; 124: 804-812.
6. Al Fayez MF et al. Transepithelial versus epithelium-off corneal collagen cross-linking for progressive keratoconus: a prospective randomized controlled trial. Cornea 2015; 34 Suppl 10:S53-6.

The analysis of pertinent data from the above articles provided the following results:

- **354 cases** have been treated by standard corneal cross-linking showing an average K_{max} change of **-1.41±1.71 D after 1 year follow-up**; the average K_{max} change was **-0.89±0.60 D at 6-month follow-up**.
- **91 cases** have been treated by transepithelial corneal cross-linking showing an average K_{max} change of was **-0.32±1.55 D after 1 year follow-up**; the average K_{max} change was **-0.52±1.20 D at 6-month follow-up**.
- In **122 control, no treated cases**, the natural history of keratoconus progression has shown a mean change of K_{max} **+0.89±2.70 D during 1-year follow-up**; the average K_{max} change was +**0.93±1.35 D at 6-month follow-up**.