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Assessment of the Predictive Ability of Theranostics for Corneal Cross-linking in Treating Keratoconus

A Randomized Clinical Trial

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Purpose: To validate the ability of theranostic imaging biomarkers in assessing corneal cross-linking (CXL) efficacy in flattening the maximum keratometry (K_{max}) index.

Design: Prospective, randomized, multicenter, masked clinical trial (ClinicalTrails.gov identifier, NCT05457647).

Participants: Fifty patients with progressive keratoconus.

Intervention: Participants were stratified to undergo epithelium-off (25 eyes) and epithelium-on (25 eyes) CXL protocols using an ultraviolet A (UV-A) medical device with theranostic software. The device controlled UV-A light both for performing CXL and assessing the corneal riboflavin concentration (*riboflavin score*) and treatment effect (*theranostic score*). A 0.22% riboflavin formulation was applied onto the cornea for 15 minutes and 20 minutes in epithelium-off and epithelium-on protocols, respectively. All eyes underwent 9 minutes of UV-A irradiance at 10 mW/cm^2.

Main Outcome Measures: The primary outcome measure was validation of the combined use of theranostic imaging biomarkers through measurement of their accuracy (proportion of correctly classified eyes) and precision (positive predictive value) to classify eyes correctly and predict a K_{max} flattening at 1 year after CXL. Other outcome measures included change in K_{max}, endothelial cell density, uncorrected and corrected distance visual acuity, manifest spherical equivalent refraction and central corneal thickness 1 year after CXL.

Results: Accuracy and precision of the theranostic imaging biomarkers in predicting eyes that had >0.1 diopter (D) of K_{max} flattening at 1 year were 91% and 95%, respectively. The K_{max} value significantly flattened by a median of −1.3 D (IQR, −2.11 to −0.49 D; $P < 0.001$); both the uncorrected and corrected distance visual acuity improved by a median of −0.1 logarithm of the minimum angle of resolution (logMAR; IQR, −0.3 to 0.0 logMAR [$P < 0.001$] and −0.2 to 0.0 logMAR [$P < 0.001$], respectively). No significant changes in endothelial cell density ($P = 0.33$) or central corneal thickness ($P = 0.07$) were noted 1 year after surgery.

Conclusions: The study demonstrated the efficacy of integrating theranostics in a UV-A medical device for the precise and predictive treatment of keratoconus with epithelium-off and epithelium-on CXL protocols. Concentration of riboflavin and its UV-A light mediated photoactivation in the cornea are the primary factors determining CXL efficacy.

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Riboflavin and ultraviolet A (UV-A) corneal cross-linking (CXL) has been recognized widely as the first treatment option to slow down or halt progression of keratoconus. Nevertheless, the procedure has shown a huge variation in efficacy (where efficacy has been determined as stabilization or flattening of the maximum keratometry [K_{max}] point value at the 1-year follow-up visit), with failure rates of various CXL protocols that fluctuated between 10% and 90%.[1–5] This disparity in outcomes highlighted the need for further research and understanding to improve the consistency and predictability of CXL for treatment of progressive keratoconus.

Mathematical models and experimental studies have supported the hypothesis that the concentration of riboflavin

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in the cornea before UV-A light irradiation is the most important variable influencing the therapeutic effect of CXL treatment.[6–8] The main mechanism of the riboflavin and UV-A CXL procedure has been shown to be the direct interaction between riboflavin triplets and reactive groups of stromal proteins.[8,9] This interaction leads to the cross-linking of stromal proteins through radical reactions, ultimately generating the therapeutic benefit of the procedure. This means that, in the ambient environment, the amount of riboflavin in the cornea and the role of a type I mechanism in the formation of additional chemical bonds between stromal proteins are predominant.[10–14] Based on this knowledge, authors have theorized that monitoring of the spatial distribution of corneal riboflavin concentration would be beneficial to overcome current limitations in treating keratoconus with CXL protocols.[6,7,9]

Theranostics is an emerging approach of personalized, predictive, and precision medicine; the term refers to the use of simultaneous imaging diagnostics for developing targeted therapies. In the field of CXL treatment, UV-A light-triggered theranostics consists of the energy excitation of the photoactivatable riboflavin, which can react to light irradiation sensitively, and thus induce controlled therapy and imaging of the cornea. In preclinical studies,[15–19] this therapeutic approach has been demonstrated to accurately predict the biomechanical stiffening effect induced on donor eye bank human corneal tissues treated either by epithelium-off or epithelium-on CXL protocols.[17–20] Predictive personalization of CXL treatment effect has been achieved by using 2 imaging biomarkers, referred to as riboflavin score and theranostic score. These biomarkers are generated by a theranostic UV-A device that processes and analyses the fluorescence emitted by the cornea in real time during CXL treatment. The riboflavin score reflects riboflavin stromal concentration, whereas the theranostic score indicates treatment effect. Through preclinical validation, the combined use of these theranostic imaging biomarkers, coupled with machine learning techniques, has improved significantly the accuracy and precision of predicting the biomechanical stiffening of human donor corneal tissue induced by CXL. The predictive capability has advanced from 79% to 94%.[15–19]

The hypothesis of the **A**ssessment of The**r**anostic **G**uided Rib**o**flavin/UV-A Corneal Cross-linking for Treatment of Keratoconus (ARGO) clinical trial focused on investigating whether the amount of riboflavin concentration in the cornea and its effective UV-A light-mediated photoactivation are the main factors influencing CXL treatment efficacy in human eyes. The study explored whether the combined use of the theranostic imaging biomarkers riboflavin score and theranostic score, calculated in real time by the investigational UV-A medical device, could classify eyes and predict efficacy of CXL treatment accurately in halting keratoconus progression (intended as a K_{max} flattening at 1 year after surgery), regardless of treatment protocol variations and regardless of whether they involved removing the corneal epithelium. The validation of the theranostic imaging biomarkers involved recording these values at the time of CXL treatment with the theranostic UV-A device, which did not offer specific outcome guidance to the investigators. Moreover, operators were unable to modify the application time of riboflavin or the UV-A treatment settings to prevent introducing additional treatment variables beyond the patient's cornea to be treated and the two CXL protocol variations.

Methods

The ARGO trial was a randomized clinical trial conducted in 3 university centers in Italy (University of Catanzaro, University of Florence, and University of Messina). The clinical study was planned in conformity with article 62 and Annex XV of European Union Regulation 2017/745 and complied with the ethical principles originating in the Declaration of Helsinki and the Convention of Oviedo. The study was conducted in accordance with Good Clinical Practice, including the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Guideline for Good Clinical Practice E6; Directive 2001/20/EC; International Organization for Standardization 14155:2011; European Union Regulation 536/2014 of the European Parliament and of the Council of April 16, 2014; and the ministerial decree of the Italian Minister of Health of August 2, 2005, decree number 211 of June 24, 2003, and the ministerial decree of July 14, 2009. The study was registered at ClinicalTrials.gov with registration number NCT05457647 and was approved by the Italian Ministry of Health (protocol no. DGDMF/I.5.i.m.2/2021/2024 05/01/2022), and ethical approval was granted by the ethics committees of Regione Calabria Sezione Area Centro (protocol no. 358 18/11/2021), AOU Gaetano Martino (protocol no. 101 23/11/2021), and Area Vasto Centro (protocol no. 21250_spe 18/01/2022).[21]

Study Protocol

Patients between 18 and 40 years of age with a confirmed diagnosis of progressive keratoconus were invited to participate in this study. According to the medical literature,[22] the primary criterion to determine disease progression was based on providing $\geq$2 Placido disc corneal topography measurements showing $\geq$1.00 D of K_{max} steepening in the year before study enrolment. The exclusion criteria were a corneal apex steeper than 63.0 D, central corneal thickness (CCT) thinner than 400 μm, corneal scarring, descemetocele, history of herpetic keratitis, concomitant eye diseases, inflammatory eye diseases, pregnancy, and breast feeding. Written informed consent was obtained from each eligible participant before enrolment.

The study consisted of a study arm receiving either an epithelium-off CXL or an epithelium-on CXL treatment protocol. Only 1 eye of each participant was designated as the study eye; if both eyes of a participant were eligible, the eye with lower corrected distance visual acuity (CDVA) was chosen as the study eye. Eligible participants were stratified with allocation ratio 1:1 into either treatment protocol using a computer-generated stratification plan with blocks. Two different blocks were created, which included eyes with a K_{max} index steeper or flatter than 54.0 D, to allocate patients with comparable baseline K_{max} values in either treatment protocol. The stratification code was given to study staff of each local site of investigation by the central monitoring site of the sponsor after the participant was considered eligible for the study and signed the informed consent form.

Participants were evaluated at baseline (visit 1; Fig 1) and at 1 week and 1, 3, 6, and 12 months after treatment (visits 2–6; Fig 1). A tabulated summary of the study was described in a previous report.[20] At each visit, participants underwent the following examinations: corneal curvature (K_{max} index) measurement, CCT

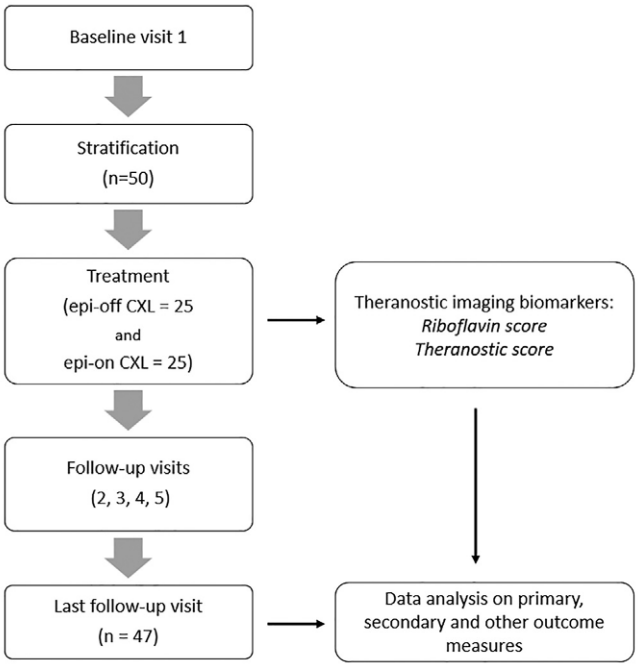

Figure 1. Diagram showing study design. Participants were stratified to undergo the epithelium-off or epithelium-on corneal cross-linking (CXL) protocol to assess reliability of the theranostic imaging biomarkers in predicting treatment efficacy, regardless of variations in riboflavin delivery protocol. The riboflavin score and theranostic score were recorded by the investigational ultraviolet A device at the time of CXL treatment; registration of these biomarkers did not influence the operators in performing treatment. Data analysis was performed on the per-protocol population, which comprised a subset of the study population, who completed ≥66% of all the visits (i.e., 4 of 6 total visits) and the last at 12 months. epi = epithelium.

measurement, endothelial cell density (ECD) measurement, uncorrected distance visual acuity (UDVA), CDVA, manifest spherical equivalent refraction (MSER), and slit-lamp biomicroscopy of the anterior segment of the eye. Ocular tonometry and dilated fundus ophthalmoscopy were performed only at the baseline and 12-month follow-up visits.

This was an observer-masked and biostatistician-masked clinical trial; the case report form was anonymized, including only the stratification code of the participant. The K_{max} (in diopters) and CCT (in micrometers) data were obtained using Placido disc keratoscopy (code BD/RDM 214431; C.S.O. srl) and Scheimpflug imaging tomography (code BD/RDM 107017; Oculus Optikgeräte GmbH), respectively. To improve the reliability of corneal curvature measurements, a minimum of 3 acquisitions were performed for each eye at each visit. If the K_{max} value varied by 1% or more between the scans, then a further scan was obtained to minimize performance bias and maximize measurement repeatability in keratoconic eyes.[23,24] The best scan then was selected for analysis. The UDVA (in logarithm of the minimum angle of resolution units, logMAR), CDVA (logMAR), and MSER (in diopters) were obtained using an ETDRS chart under photopic conditions at a test distance of 4 m. The ECD (in cells per square millimeter) was obtained using specular microscopy.

Contact lens wearers were instructed to discontinue their use for a minimum of 3 weeks before the preoperative eye examination. In addition, these patients were asked to discontinue the use of contact lenses during follow-up to avoid methodologic bias during the study.

Intervention

The C4V CHROM04VIS (code BD/RDM 2103580; Regensight srl) is an active medical device that delivers a homogeneous circular spot of ultraviolet light (365-nm wavelength) onto the cornea with indication of use of treating pathologic conditions of the cornea with a CXL procedure. The emitted UV-A light radiation and energy dose used both for imaging and treatment are controlled continuously by optical and electronic means during operation. In this trial, the UV-A medical device was equipped with a theranostic software module, as previously described.[18,19]

All treatments were performed under topical anesthesia; 0.4% oxybuprocaine hydrochloride eye drops were instilled 4 times over 20 minutes before treatment. At the beginning of the CXL treatment, after placing a lid speculum, the operator was instructed to align and focus the Placido rings, which were projected by the active medical device onto the cornea through the interaction with an augmented reality graphic user interface displayed on the touchscreen.[18–20] This allowed for precise focusing of the UV-A light onto the cornea in the next CXL treatment steps. As soon as focusing was completed, the operator was invited to acquire an image of the cornea to treat by pressing the footswitch to activate a 3-mW/cm^2 UVA light irradiance for 2 seconds. The measure was recorded as the baseline corneal fluorescence signal from the UVA theranostic device. Thereafter, in participants undergoing the epithelium-off CXL protocol, the central 9-mm corneal epithelium was removed using a blunt spatula (code JD2112/10; E. Janach srl); whereas in participants undergoing the epithelium-on CXL protocol, a sterile Biopore membrane attached to a plastic cylinder (code PICM01250; Merck KGaA) was pressed for 2 seconds against the central cornea to remove the precorneal mucin layer, according to the methodology validated in previous randomized controlled trial (ClinicalTrials.gov identifier, NCT02117999).[25]

A hypotonic 0.22% riboflavin ophthalmic solution (code BD/RDM 2112472; Ritsight, Regensight srl) was applied on the cornea before therapeutic UV-A light irradiation in all participants. Application of riboflavin eye drops was carried out according to

manufacturer's instruction for use every 20 seconds for 15 minutes and for 20 minutes in the epithelium-off and epithelium-on CXL protocols, respectively. As soon as the imbibition phase was completed, the cornea was irradiated by 10 mW/cm^2 UVA power density for 9 minutes (5.4 J/cm^2 energy density) with a 7.0-mm irradiation beam diameter in all participants; no riboflavin was applied over the corneal surface during UV-A light irradiation in any patient.

At preset intervals, both during the imbibition and UV-A phototherapy phases, the UV-A theranostic device recorded the fluorescence images emitted by the cornea to estimate the riboflavin concentration and treatment efficacy, respectively.[18,19] The theranostic measurements were performed over a 3.0-mm central area of the cornea by irradiating the cornea under treatment with 3 mW/cm^2 UV-A power density for 2 seconds (as for baseline measure); the theranostic device processed corneal fluorescence images and provided the operator with an online measure estimating the corneal riboflavin concentration (the riboflavin score) and a calculated value estimating treatment efficacy (the theranostic score, which was calculated during the ultraviolet phototherapy phase only).[18–20] The riboflavin score was calculated in real time by the theranostic device, taking into consideration the CCT value of the cornea to treat and using a second-order polynomial function that depends on the green intensity signal acquired by the red, green, blue camera of the theranostic device[15,16]; it is expressed in dimensionless units. In the living eye, its value ranges from 0.00 to 2.70, with a resolution of ± 0.05. The theranostic score was calculated using a polynomial function that depends on the green intensity signals emitted by the cornea tissue before UV-A phototherapy (i.e., the last measurement of the dosing phase) and during the UV-A phototherapy; its value, expressed in a dimensionless unit, ranges from 0.00 to 1.50, with a resolution of ± 0.05.[18,19] A full description of the main treatment steps of investigational CXL procedures was given in previous reports.[18–20] It is important to note that in this clinical trial, the theranostic UV-A device did not provide preferential outcome measures to the operator (e.g., it did not alert the operator to stop or continue riboflavin application until the preset time had elapsed), who also were unaware about the cutoff values of both the riboflavin score and theranostic score. In addition, both the application time of riboflavin and UV-A treatment settings could not be changed by operators. This process was established to validate the theranostic software module without introducing treatment variables other than the patient's cornea to be treated and the 2 CXL protocol variations (i.e., epithelium-off and epithelium-on).

At the end of CXL treatment, a bandage contact lens was applied onto the cornea of all patients; it remained in place until the first scheduled postoperative visit at 7 days to allow the corneal epithelium to heal as well as to mask the observer from the type of CXL protocol and to minimize performance bias. All patients were prescribed topical antibiotic (0.3% ofloxacin eyedrops) 4 times daily for 7 days, corticosteroid (0.2% fluorometholone eyedrops) twice daily for 21 days, and lubricant eye drops (0.2% sodium hyaluronate eyedrops) 4 times daily for 3 months.

Outcome Measures

The primary outcome measure was the validation of the combined use of the theranostic imaging biomarkers (see "Statistical Analysis"). True-positive results, false-positive results, true-negative results, and false-negative results were evaluated 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 (in diopters) by >0.1 D at 12 months after surgery. Receiver operating characteristic curve analysis was used to evaluate the cutoff values of the theranostic imaging biomarkers and to determine the area under the receiver operating characteristic (ROC) curve.

The secondary outcome measure of efficacy was assessed by measuring changes in K_{max} value (in diopters) from baseline to 12 months after surgery. The secondary outcome measure of safety was assessed by measuring change in ECD (in cells per square millimeter, cells/mm^2) in the same period. Other outcome measures included: change in UDVA (logMAR) at 12 months after surgery, change in CDVA (logMAR) at 12 months after surgery, change in MSER (in diopters) at 12 months after surgery, and change in CCT (in micrometers) at 12 months after surgery. The analysis of data in stratification groups was performed as exploratory outcome of the study.

Adverse Events

Any adverse event (AE) occurring in a participant enrolled in the clinical trial was reported in detail on the case report form according to the ICH Guidelines and the Medical Device Coordination Group 2020-10-2 Guidance safety report form. The Medical Dictionary for Regulatory Activities was used to code all AEs. Corneal haze was graded on a scale (grade, 0–4), as typically used after photorefractive keratectomy.[26]

Sample Size Calculation

Based on the analysis of literature data and the expected efficacy of CXL showing an average K_{max} change of −1.0 ± 0.8 D at the 1-year follow-up visit after treatment[1–5,20] and assuming a response rate of 50% of patients who could reach the threshold of 1.0 D of K_{max} flattening at 12 months after CXL, a minimum sample size of 42 participants and eyes achieved 91% power in detecting a difference of 0.25 between the area under the receiver operating characteristic curve of the null hypothesis of 0.60 and the alternative hypothesis of 0.85 using a 2-sided z test at a significance level of 0.05. Considering a 20% or less dropout rate, 50 participants (25 per treatment protocol) were allowed to be enrolled in the study.

Statistical Analysis

Numerical variables were summarized as mean and standard deviation and as median and interquartile range (IQR). Categorical variables were represented as frequencies and proportions. For numerical variables, a paired Wilcoxon signed-rank test was used to compare the distributions before surgery and 12 months after surgery. Bonferroni correction was applied to analysis of exploratory outcome measures of stratification groups. For all reported AEs, the data were presented with events listed in order of decreasing frequency; no statistical analysis was conducted on the AE data.

The optimal cutoff values in predicting the propensity of CXL treatment to flatten the K_{max} value at 12 months was determined based on the results of preclinical studies,[15,18,19] where a riboflavin score of >0.4 and a theranostic score of 0.60 or more were associated with a significant biomechanical stiffening of the human cornea (measured with either atomic force microscopy or dynamic air-puff tonometry) treated by CXL.[15–19] For the in vivo study, these specific cutoff values were based on the assumption of a correlation between the corneal stiffness and the corneal curvature, as described in previous clinical reports.[27–29] The accuracy and precision (95% confidence interval [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, respectively. The miss

rate, which is the probability that a true-positive result is missed by the theranostic methodology, was determined by calculating the false-negative rate. 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 >0.1 D of K_{max} flattening) at 1 year in the study population who participated in and completed ≥66% of all the visits (i.e., 4 of 6 total visits) and the last at 12 months (i.e., per-protocol population; Fig 1). These criteria served as benchmarks to assess the success of the study and the efficacy of CXL treatment in halting the progression of the disease.

Statistical significance was set at 0.05. All the analyses were performed using the statistical software R (latest version available; R Foundation for Statistical Computing).

Table 1. Patient Demographics and Medical Histories

Variable	Data
No.	50
Sex, no. (% female)	13 (26)
Age (yrs)	
Mean ± standard deviation	26 ± 5
Median (interquartile range)	26 (22–30)
Positive family history of keratoconus, no. (%)	12 (24)
Concurrent pathologic features, no. (%)	
History of allergy/dermatitis	4 (8)
Hypothyroidism	3 (6)
Diabetes mellitus	1 (2)
Systemic lupus erythematosus and ulcerative colitis	1 (2)

Results

Participant Baseline Demographics and Measures

Fifty patients (50 eyes), with a mean age of 26 ± 5 years, were enrolled in the ARGO trial; of these, 37 patients were male, and 13 patients were female. Twelve participants (24%) had a positive family history of keratoconus. Participant demographics are presented in Table 1. At baseline, the average K_{max} value was 55.5 ± 4.0 D; disease progression was determined by comparing this baseline value with the K_{max} value measured by 1 year before participation in the clinical trial, the mean ± standard deviation of which was 53.8 ± 3.8 D ($P = 0.03$).

Intervention Measures

All CXL treatments were uneventful; at 7 days, the corneal epithelium was completely healed in all participants. No protocol deviation was recorded during follow-up. The riboflavin score and the theranostic score on average were 1.23 ± 0.68 (median, 1.2; IQR, 0.6–1.7) and 0.93 ± 0.19 (median, 0.90; IQR, 0.8–1.0), respectively (Fig 2). Forty-seven patients completed ≥66% of scheduled visits and the last visit at 12 months; their clinical data were used for statistical analysis on primary, secondary, and exploratory clinical outcomes. Two participants dropped out at 3 months after surgery because of their inability to adhere to the study visit schedule and the distance of the university clinics from their home town.

Primary Outcome Measure

Accuracy of the theranostic imaging biomarkers generated by the UV-A device to predict the propensity of CXL in flattening the K_{max} value at 12 months was 91% (95% CI, 79%–98%). The algorithm was effective to predict the clinical outcome in 43 of 47 eyes (22/24 eyes and 21/23 eyes in epithelium-off and epithelium-on CXL protocols, respectively); the algorithm precision was 95% (95% CI, 85%–99%), and the miss rate was 3%. The area under the receiver operating characteristic curve was 0.73 (95% CI, 0.56–0.87). Figure 3 illustrates the median changes in K_{max} for eyes that met the defined cutoff values for both theranostic imaging biomarkers (identified as true-positive results) during follow-up. The primary data outcomes in the study population are summarized in Table 2. Two patients with true-negative findings (patients ON08 and ON10; Table 3), who underwent the epithelium-on CXL protocol, showed a riboflavin score of ≤0.40. Two patients with false-negative findings (patients ON02 and ON09; Table 3) showed a riboflavin score of 0.38, which was close to the cutoff value (the measurement error of the method is ± 0.05). Two patients with false-positive findings (patients OFF12 and OFF13; Table 3) were recorded after the epithelium-off CXL protocol; in these participants, the mean change of K_{max} was +0.10 ± 0.01 D at 12 months, whereas progression of K_{max} was +2.11 ± 0.84 D in the year preceding enrolment in the clinical study.

Secondary Outcome Measures

Table 4 summarizes the clinical data outcome (showing both mean ± standard deviation and median and IQR values) in the study population during follow-up. The efficacy of CXL treatment was determined by measuring the K_{max} change; at 12 months, the median K_{max} value flattened significantly by −1.3 D (IQR, −2.11 to −0.49 D; $P < 0.001$). The longitudinal changes of K_{max} values during follow-up are shown in Figure 4. Twenty-eight eyes (60%) showed >1.0 D of K_{max} flattening at 12 months, and no eye showed >0.5 D of K_{max} steepening in the same period.

The safety of the CXL treatment was determined by assessing ECD. At 12 months, the distribution of ECD was unchanged with respect to that before surgery (median change, −63 cells/mm^2; IQR, −230 to 146 cells/mm^2; $P = 0.33$). Both UDVA (median change, −0.1 logMAR; IQR, −0.3 to 0.0 logMAR; $P < 0.001$) and CDVA (median change, −0.1 logMAR; IQR, −0.2 to 0.0 logMAR; $P < 0.001$) improved significantly by 1 EDTRS line. Nineteen eyes (40%) and 12 eyes (25%.) gained 2 or more UDVA and CDVA ETDRS lines, respectively, whereas no eye lost ≥2 UDVA or CDVA EDTRS lines. The changes in ECD and visual acuity over the follow-up period are shown in Figures 5 and 6, respectively. At 12 months, the CCT value was unchanged compared with that before surgery (median change, −4 μm; IQR, −13 to 2 μm; $P = 0.07$). The 12-month MSER also was unchanged compared with that obtained before surgery (median change, 0.0 D; IQR, −0.50 to 0.62 D; $P = 0.519$).

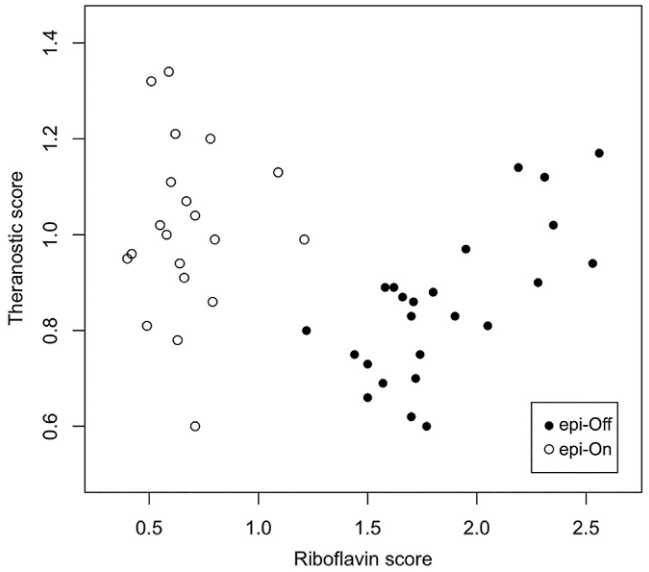

Figure 2. Scatterplot showing riboflavin score and theranostic score recorded in the analyzed study population (n = 47). Observing the distribution of points categorized based on whether eyes were treated with the epithelium-off or epithelium-on corneal cross-linking (CXL) protocol helped to identify any patterns or differences between the two treatment approaches. These visual representations can assist in further understanding the relationships between the variables measured during the CXL procedure and their impact on treatment outcomes. Predictive personalization of CXL therapeutic benefit relied on achieving predetermined cutoff values for both riboflavin score and theranostic score during treatment. The operators were unaware of these cutoff values at the time of surgery. epi = epithelium.

AEs

Corneal haze was the only AE recorded during follow-up. It was found in 3 eyes (6%; n = 3 eyes with grade 1 haze) at 7 days, in 6 eyes (13%; n = 5 eyes with grade 1 haze; n = 1 eye with grade 0.5 haze) at 1 month, in 3 eyes (6%; n = 2 eyes with grade 1 haze; n = 1 eye with grade 0.5 haze) at 3 months, and in 3 eyes (6%; n = 3 eyes with grade 0.5 haze) at 6 and 12 months. Corneal haze occurred in eyes treated by the epithelium-off CXL protocol, except for 1 patient who underwent epithelium-on CXL (patient ON14) recorded at 12 months. No changes in UDVA and CDVA were recorded in the 3 eyes and participants with corneal haze at 12 months with respect to the measurements obtained at the baseline visit.

Exploratory Outcome Measures in Stratification Groups

In the epithelium-off CXL protocol, the riboflavin score was 1.84 ± 0.35 (median, 1.74; IQR, 1.62−2.05), and the theranostic score was 0.85 ± 0.15 (median, 0.86; IQR, 0.75−0.90); in the epithelium-on CXL protocol, the scores were 0.62 ± 0.21 (median, 0.62; IQR, 0.49−0.71) and 1.01 ± 0.18 (median, 1.0; IQR, 0.91−1.13), respectively.

The tabulated clinical data of the two stratification groups were added as supplementary material (Tables S5 and S6, available at www.aaojournal.org). At 12 months, the distribution of K_{max} values significantly flattened by a median change of −1.2 D (IQR, −2.4 to −0.5 D; $P < 0.001$) and −0.90 D (IQR, −1.9 to −0.3 D; $P < 0.001$) after the epithelium-off and epithelium-on CXL protocols, respectively. Both the UDVA and CDVA significantly improved after both CXL protocols; the median UDVA change was −0.1 logMAR (IQR, −0.2 to 0.0 logMAR; $P = 0.038$) and −0.1 logMAR (IQR, −0.3 to 0.00 logMAR; $P = 0.003$) after the epithelium-off and epithelium-on CXL protocols, respectively, and the median CDVA change was −0.1 logMAR (IQR, −0.2 to 0.0 logMAR; $P = 0.004$) and −0.1 logMAR (IQR, −0.2 to 0.0 logMAR; $P < 0.001$), respectively.

The ECD was stable in both CXL protocols; the median change was −82 cells/mm^2 (IQR, −206 to 139 cells/mm^2; $P = 1.000$) and −43 cells/mm^2 (IQR, −249 to 146 cells/mm^2; $P = 1.000$) 12 months after the epithelium-off and epithelium-on CXL protocols, respectively. The CCT thinned significantly solely after the epithelium-off CXL protocol (median change, −12 μm; IQR, −25 to 0 μm; $P = 0.004$), whereas it was steady after the epithelium-on CXL protocol (median change, +4 μm; IQR, −4 to 6 μm; $P = 0.364$) at 12 months after surgery. No significant change in the median MSER values of either stratification group was noted from baseline to 12 months after surgery (median, 0.00 D [IQR, −0.62 to 0.94 D; $P = 1.000$] after epithelium-off CXL and median, 0.00 D [IQR, −0.44 to 0.44 D; $P = 1.000$] after epithelium-on CXL).

Discussion

In this multicenter, randomized clinical trial, the accuracy and precision of the combined use of the theranostic imaging biomarkers in predicting a K_{max} flattening of >0.1 D at 12 months after surgery were 91% and 95%, respectively. The results highlighted the efficacy of using these biomarkers to predict therapeutic outcomes reliably for each

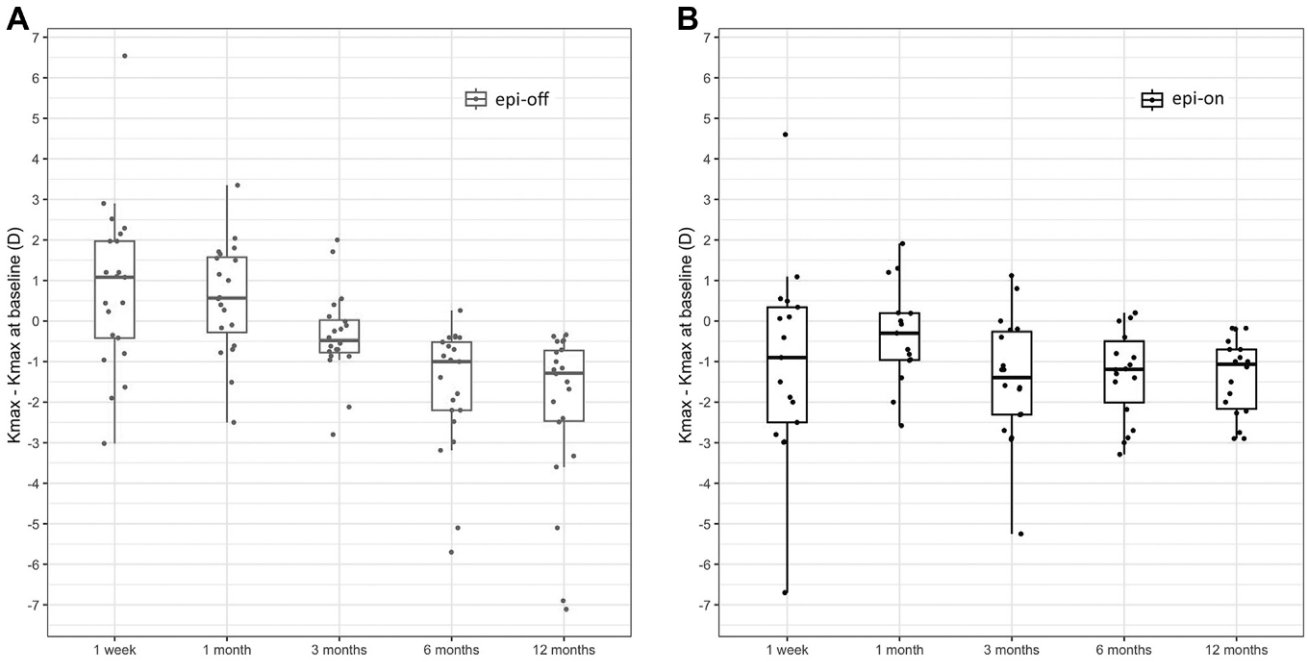

Figure 3. Boxplots with jittered data points showing the median changes in maximum keratometry (K_{max}) index at each follow-up visit (1 week and 1, 3, 6, and 12 months) in comparison with preoperative median K_{max} values in patients with true-positive results. The data are presented separately for the two stratification groups: the epithelium-off corneal cross-linking [CXL] protocol (**A**) and the epithelium-on CXL protocol (**B**). The patients with true-positive results exhibited an average K_{max} flattening of −2.08 ± 1.99 diopters (D) and −1.31 ± 0.96 D after epithelium-off and epithelium-on CXL protocols at 12 months, respectively; K_{max} flattening was >0.2 D in all patients with true-positive results, except for 1 eye (patient ON17, who showed −0.15 D of K_{max} flattening). It is noteworthy that the average change in K_{max} among patients with true-positive results who underwent the epithelium-on CXL protocol was comparable with the state-of-the-art clinical outcome of epithelium-off CXL at 12 months (−1.3 ± 1.4 D[1–5,20,30–36]). epi = epithelium.

patient undergoing CXL treatment. The UV-A light-mediated theranostic strategy for CXL has shown that for clinically significant K_{max} flattening, the riboflavin score and theranostic score must be >0.4 and 0.60 or more, respectively, thus confirming the outcome of preclinical studies.[18,19] Validating the UV-A device's theranostic software module will provide valuable guidance to eye surgeons on the optimal enrichment of the cornea with riboflavin before UV-A light therapy. This validation is particularly important for reducing the risk of treatment failure in epithelium-on CXL protocols (Fig 3), ensuring more effective and safer procedures. The implementation of the clinically validated theranostic software module within the UV-A device will enable the operator to initiate UV-A light phototherapy solely when the riboflavin score surpasses the cutoff value of 0.40.

The CXL treatment performed with the innovative UV-A medical device and 0.22% riboflavin ophthalmic solution

Table 2. Predictive Ability of the Theranostic Imaging Biomarkers Produced by the Ultraviolet A Medical Device during Intervention in Assessing the Propensity of Corneal Cross-linking Treatment in Flattening the Maximum Keratometry Value at 12 Months

Theranostic Imaging Biomarkers (Cutoff Values)	Predicted Outcome: Maximum Keratometry Change at 1 Year		Total
	Flattening of >0.10 Diopter	*Flattening of ≤0.10 Diopter*	
Riboflavin score >0.40 and theranostic score ≥0.60	True-positive results (n = 41): K_{max} change, −1.72 ± 1.63 D (−2.08 ± 1.99 D after epithelium-off CXL; −1.31 ± 0.96 D after epithelium-on CXL)	False-positive results (n = 2): K_{max} change, +0.10 ± 0.01 D (epithelium-off CXL only)	43
Riboflavin score ≤0.40, theranostic score <0.60, or both	False-negative results (n = 2): K_{max} change, −0.52 ± 0.26 D (epithelium-on CXL only)	True-negative results (n = 2): K_{max} change, +0.23 ± 0.25 D (epithelium-on CXL only)	4
Total	43	4	47

CXL = corneal cross-linking; D = diopter; K_{max} = maximum keratometry.

Table 3. Clinical Data in Sample Population Patients with False-Positive Findings (Maximum Keratometry Flattening <0.10 Diopter, with Riboflavin Score and Theranostic Score of >0.40 and ≥0.60, respectively) and False-Negative Findings (Maximum Keratometry Flattening >0.10 Diopter, with Riboflavin Score and Theranostic Score of ≤0.40 and <0.60, respectively)

Patient Identification (Sex; Age [yrs])	Maximum Keratometry (Diopters)		Preoperative Central Corneal Thickness (μm)	Preoperative Manifest Spherical Equivalent Refraction (Diopters)	Riboflavin Score	Theranostic Score	Change Maximum Keratometry (D) at 12 Months
	1 Year before Surgery	*Before Surgery*					
False-positive findings							
OFF12 (M; 20)	47.8	49.3	529	−1.00	1.50	0.73	+0.09
OFF13 (M; 33)	53.3	56.0	500	+0.13	2.19	1.14	+0.10
False-negative findings							
ON02 (F; 28)	46.6	48.9	479	−1.00	0.38	1.20	−0.33
ON09 (F; 23)	51.7	58.3	426	−3.00	0.38	0.70	−0.70

F = female; M = male.

demonstrated significant reliability; at 12 months, the average K_{max} significantly flattened by 1.5 ± 1.6 D; 28 of 47 eyes (60% of total) showed K_{max} flattening of >1.0 D, thus confirming that power sampling of the trial was appropriate and that clinically significant conclusions could be driven from this study.[20] No changes in ECD were recorded at 12 months in comparison with those recorded before surgery; the clinically significant average UDVA and CDVA improvement in the study population provided further clinical evidence of the benefit of CXL in the treatment of patients with progressive keratoconus. Only 1 AE, such as corneal haze, was recorded during follow-up; at 12 months, grade 0.5 corneal haze was found in 6% of the study population (n = 2 patients undergoing epithelium-off CXL and n = 1 patient undergoing epithelium-on CXL).

Patients were stratified randomly into groups to undergo either the epithelium-off or epithelium-on CXL protocol; this was done to assess the reliability of the theranostic imaging biomarkers in predicting treatment efficacy, regardless of variations in riboflavin delivery protocol, which has been found to affect clinical outcomes significantly.[1–5] It was outside the scope of this trial to compare findings between the epithelium-off and epithelium-on CXL protocols; however, it was noteworthy that the efficacy outcome found after the epithelium-on CXL protocol (average K_{max} flattening at 12 months, −1.1 ± 1.0 D) was 4 times more effective than the current clinical outcome of epithelium-on CXL treatment (−0.3 ± 1.5 D).[1–5,20,30–34] Data from the epithelium-off CXL stratification group were consistent with those already published in

Table 4. Clinical Data Outcomes of the Study Population (Per Protocol Population) during 12 Months of Follow-up

Variable	Baseline (n = 47)	1 Week (n = 47)	1 Month (n = 47)	3 Months (n = 47)	6 Months (n = 47)	12 Months (n = 47)
K_{max} (D)						
Mean ± SD	55.5 ± 4.0	55.4 ± 4.5	55.7 ± 4.1	54.7 ± 4.1	54.1 ± 4.1	54.0 ± 4.0*
Median (IQR)	56.0 (52.9–57.9)	56.3 (51.5–58.1)	56.2 (53.3–58.5)	54.8 (51.9–57.2)	54.6 (50.3–56.7)	54.7 (51.4–56.5)
ECD (cells/mm^2)						
Mean ± SD	2686 ± 331	2583 ± 341	2532 ± 339	2615 ± 285	2643 ± 310	2624 ± 335
Median (IQR)	2691 (2472–2883)	2693 (2467–2780)	2521 (2381–2707)	2675 (2441–2753)	2655 (2441–2795)	2645 (2431–2853)
CCT (μm)						
Mean ± SD	489 ± 29	495 ± 43	477 ± 31	473 ± 32	479 ± 33	484 ± 32
Median (IQR)	487 (470–512)	492 (468–517)	475 (458–495)	474 (456–495)	482 (452–503)	484 (464–511)
UDVA (logMAR)						
Mean ± SD	0.50 ± 0.37	0.49 ± 0.32	0.42 ± 0.33	0.41 ± 0.31	0.38 ± 0.31	0.36 ± 0.32*
Median (IQR)	0.4 (0.2–0.8)	0.5 (0.3–0.7)	0.4 (0.2–0.7)	0.4 (0.1–0.6)	0.4 (0.2–0.5)	0.3 (0.1–0.5)
CDVA (logMAR)						
Mean ± SD	0.18 ± 0.18	0.23 ± 0.18	0.20 ± 0.18	0.14 ± 0.16	0.11 ± 0.15	0.08 ± 0.14*
Median (IQR)	0.1 (0.1–0.2)	0.2 (0.1–0.4)	0.1 (0.1–0.4)	0.1 (0.0–0.2)	0.1 (0–0.2)	0.0 (0–0.1)
MSER (D)						
Mean ± SD	−1.71 ± 2.06	−1.78 ± 2.38	−1.60 ± 2.28	−1.41 ± 2.24	−1.41 ± 2.18	−1.54 ± 2.25
Median (IQR)	−1.25 (−3.0 to 0.0)	−1.25 (−3.1 to 0.0)	−1.0 (−2.8 to 0.0)	−0.8 (−2.6 to 0.0)	−0.9 (−2.6 to 0.0)	−1.25 (−2.5 to 0.0)

CCT = central corneal thickness; CDVA = corrected distance visual acuity; D = diopter; IQR = interquartile range; logMAR = logarithm of the minimum angle of resolution; MSER = manifest spherical equivalent refraction; SD = standard deviation; UDVA = uncorrected distance visual acuity.
*$P < 0.001$.

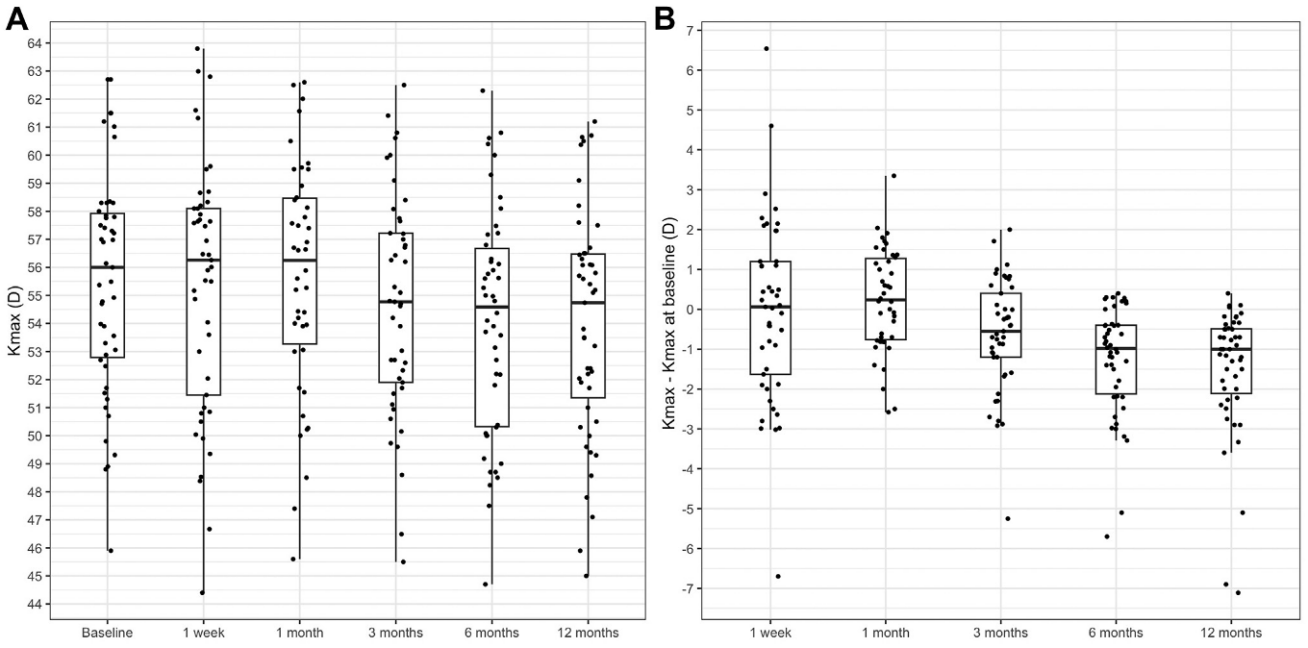

Figure 4. **A**, Stratified boxplots with jittered data points showing a detailed representation of the data distribution for the corneal topography maximum keratometry (K_{max}) index change in the study population during follow-up. **B**, Boxplot with jittered data points showing the median changes in K_{max} index at each follow-up visit (1 week and 1, 3, 6, and 12 months) in comparison with preoperative median K_{max} values. The study identified a notable flattening of K_{max} starting at 3 months after surgery, with a further improvement at 6 months, followed by stable values in final follow-up visit at 12 months. At 6 and 12 months, most data points exhibited a notable skew toward negative values (i.e., more K_{max} flattening). This finding demonstrated an improvement in corneal shape after CXL treatment, with stabilization persisting beyond the initial postoperative period. D = diopter.

the literature showing the time course of clinical and instrumental measures after CXL, except for the greater average K_{max} flattening at 12 months (-1.9 ± 2.0 D vs. -1.3 ± 1.4 D) found in this study.[1–5,35,36] Vision improvement was found after both CXL protocols.

Study Limitations

This study assessed CXL efficacy by measuring the change in the K_{max} index, which is aligned with commonly used approaches in the field. Nevertheless, the information provided by the K_{max} index has been shown not to be exhaustive[37,38] because it refers to only a single point in the corneal topographic map and, therefore, cannot be sensitive enough to determine the overall keratoconus corneal shape change. In this study, no eye showed a theranostic score of less than 0.60; this finding can be attributed to the investigators strictly following the treatment protocol, which specifically advised in the clinical investigation plan against applying riboflavin on the cornea during the UV-A light therapy phase. This adherence and the carefully controlled UV-A light energy dose delivered by the active device to each eye ensured the effectiveness of the treatment and benefit to patients; it has been demonstrated that a precorneal riboflavin film decreases efficiency of UV-A light-mediated photoactivation of stromal riboflavin.[8,15,18,19,39] In addition, this finding supported the evidence that the corneal epithelium does not interfere with effective UV-A light-mediated photoactivation of stromal riboflavin in vivo.[9,17–19] Because the study's objective was to validate the predictive capability of the combined use of theranostic imaging biomarkers in assessing the therapeutic efficacy of CXL treatment, this finding did not affect the study's performance. This was confirmed by the variation recorded in the riboflavin score across eyes treated with various CXL protocols, as consistently found in preclinical studies.[18,19] These laboratory studies revealed that, for effective photoactivation of riboflavin to generate cross-linking bonds among stromal proteins, an adequate amount of the substance must penetrate the corneal stroma before exposure to UV-A light. This required dosage has been found in preclinical studies to vary based on whether the epithelium remains intact (Fig 2), as reaffirmed in this clinical study.[17–20]

The list of AEs may not be fully comprehensive because the first postoperative visit was scheduled at 7 days after treatment, excluding immediate postoperative events, such as eye pain, stromal edema, corneal striae, and conjunctival hyperemia, from the assessment.[40]

Perspectives

Analyzing long-term data from patients participating in this study, along with data gathered from postmarket clinical follow-up studies (in accordance with article 61 and Annex XIV of European Union Regulation 2017/745) will strengthen the reliability of the new paradigm for the precise and effective treatment of keratoconus. With increasing use of the theranostic platform, researchers can gather more clinical data and potentially can identify specific variables

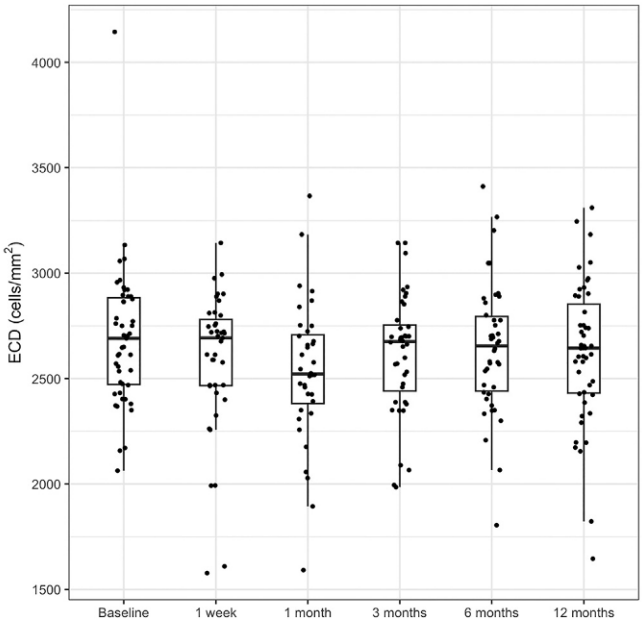

Figure 5. Stratified boxplot with jittered data points for the endothelial cell density (ECD) change in the study population during follow-up. The ECD values were almost stable along the course of the study, except for the 1-month follow-up visit, caused by corneal haze in patients undergoing the epithelium-off corneal cross-linking (CXL) procedure, which decreased the clear visualization of the corneal endothelium (see description of adverse events in text). This finding evidenced the high safety of the CXL treatment for keratoconus.

that may influence treatment outcomes, leading to improved understanding and potential refinements to the CXL treatment protocols developed through an integrated artificial intelligence algorithm of the theranostic UV-A device. For example, it is crucial to conduct a more comprehensive analysis of the response to the epithelium-off CXL protocol and to determine factors contributing to the lack of K_{max} flattening in 8% of patients (patients OFF12 and OFF13; Tables 2 and 3). In addition, at the end of application time, 17% of patients undergoing the epithelium-on CXL protocol

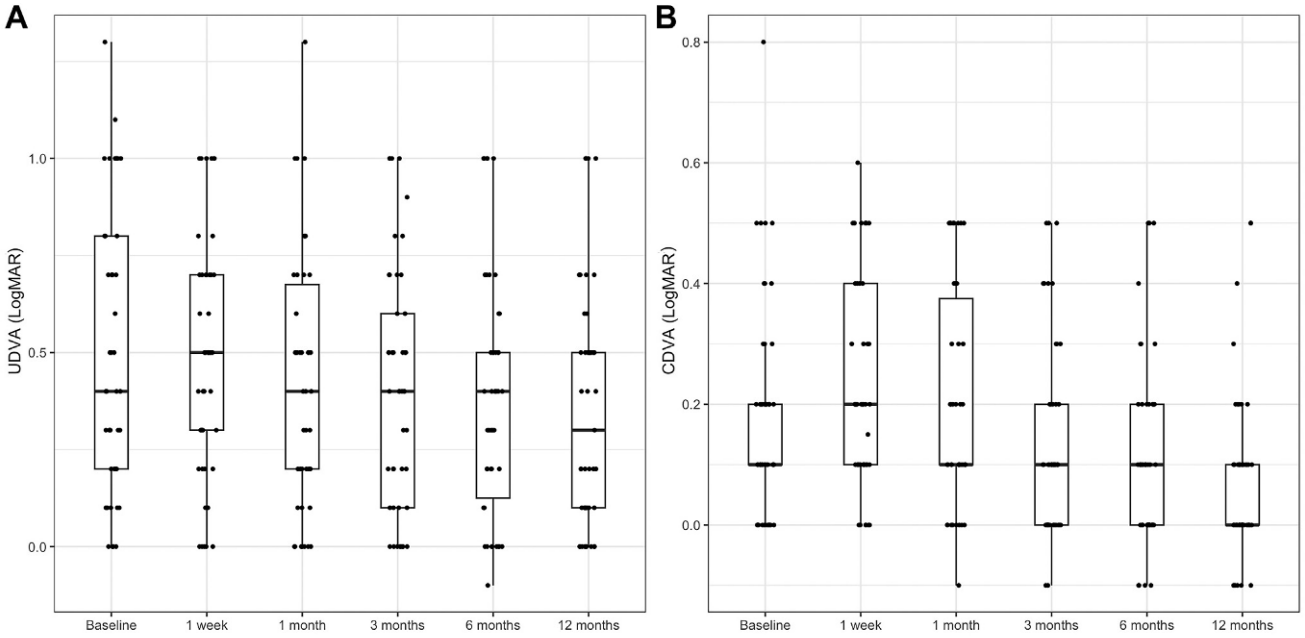

Figure 6. Stratified boxplot with jittered data points for (**A**) the uncorrected distance visual acuity (UDVA) change and (**B**) the corrected distance visual acuity (CDVA) change in the study population during follow-up. The UDVA showed the most improvement between the 6-month and 12-month follow-up visits, whereas CDVA already showed improvement by 1 month and remained stable. This finding evidenced that the corneal crosslinking treatment significantly benefited visual improvement. logMAR = logarithm of the minimum angle of resolution.

(n = 4/23; Tables 2 and 3) did not reach the cutoff value of >0.40 of riboflavin score; 2 patients (patients ON08 and ON10) did not show flattened K_{max} reading at 12 months, as expected; and 2 patients showed K_{max} flattening of >0.1 D (patients ON02 and ON09; Table 3).

Caution should be exercised when generalizing the findings of this study to other riboflavin ophthalmic solutions because of the differences in stromal permeation, both in epithelium-off and epithelium-on delivery protocols, among different products.[41,42] The riboflavin ophthalmic solution used in this clinical trial has been shown to permeate the human corneal stroma significantly more than all clinically available products in both epithelium-off and epithelium-on delivery protocols for CXL treatment.[42] The results from the laboratory investigation regarding the riboflavin score were equivalent in this study both for epithelium-off and epithelium-on delivery protocols (1.79 ± 0.07 and 0.67 ± 0.19, respectively). Further research and clinical trials are necessary to assess the efficacy and safety of other riboflavin formulations on CXL treatment outcomes using the theranostic UVA medical device.

Social Impact

Keratoconus is a chronic, progressive, and life-altering disease with considerable clinical, economic, and humanistic consequences to young patients and their families. The global disease prevalence is now estimated to be significantly higher than formerly thought (from 0.002% before the 2000s to > 1% in the 2020s).[43] An accurate and precise therapeutic approach predicting the therapeutic benefit of CXL treatment can impact positively the visual function and quality of life of patients, reducing the disease's social and economic burden.[44] Reports have shown that effective CXL can enhance the functional ability and emotional well-being in patients with keratoconus significantly.[45–48]

The combined use of theranostic imaging biomarkers generated during treatment allowed for an immediate predictive assessment of CXL treatment efficacy with high accuracy and precision, regardless of treatment protocol variations and regardless of whether they involved removing the corneal epithelium.[15,18,19] The findings from the ARGO clinical study demonstrated the promising potential of theranostics technology in maximizing the therapeutic benefit of CXL for treating progressive keratoconus with predictive personalization of outcomes.

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Footnotes and Disclosures

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Disclosure(s):

All authors have completed and submitted the ICMJE disclosures form.

The author(s) have made the following disclosure(s):

G.L.: Patent − "Process for Dosing a Chromophoric Agent in a Corneal Tissue and Apparatus for Controlling the Dose" and "Liquid Formulation, in Particular to Treat a Corneal Tissue," related to the method and system of theranostic-guided corneal cross-linking and riboflavin ophthalmic formulation, respectively; Equity owner − Regensight srl

S.S.: Equity owner − Regensight srl. M.L.: Patent − "Process for Dosing a Chromophoric Agent in a Corneal Tissue and Apparatus for Controlling the Dose" and "Liquid Formulation, in Particular to Treat a Corneal Tissue," related to the method and system of theranostic-guided corneal cross-linking and riboflavin ophthalmic formulation, respectively; Equity owner − Regensight srl

The promoter and sponsor of the study was Regensight srl. The clinical investigation was conducted in accordance with the provisions of articles 62 to 80 and Annex XV of the European Union Regulation 2017/745 as part of the clinical evaluation for conformity assessment purpose of the ultraviolet A theranostic medical device. The object of the clinical investigation was the validation of the theranostic software module of a conformité européenn (CE)-certified ultraviolet A medical device for the treatment of pathologic conditions of the cornea. The device in question conformed 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. A system for compensation for any damage sustained by a study participant has been in place in the form of insurance for the risk of clinical trial (certificate no. 390-76512553-30018). The sponsor has provided evidence that the investigation has been conducted in line with good clinical practice through internal inspection. No undue influence, including that of a financial nature, has been exerted on investigators and study participants. Study sponsorship did not influence the scientific, technical, or procedural autonomy of the investigators and study staff in conducting the clinical investigation in accordance with the approved clinical investigation plan.

HUMAN SUBJECTS: Human subjects were included in this study. Study was approved by the Italian Ministry of Health (prot. n. DGDMF/I.5.i.m.2/2021/2024 05/01/2022) and granted by the Ethics Committees of Regione Calabria Sezione Area Centro (prot. n. 358 18/11/2021), AOU Gaetano Martino (prot. n. 101 23/11/2021) and Area Vasto Centro (prot. n. 21250_spe 18/01/2022). All research adhered to the tenets of the Declaration of Helsinki. All participants provided informed consent.

No animal subjects were included in this study.

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Obtained funding: M. Lombardo

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Abbreviations and Acronyms:

AE = adverse event; **CCT** = central corneal thickness; **CDVA** = corrected distance visual acuity; **CI** = confidence interval; **CXL** = corneal cross-linking; **D** = diopter; **ECD** = endothelial cell density; **IQR** = interquartile range; **K_{max}** = maximum keratometry; **logMAR** = logarithm of the minimum angle of resolution; **MSER** = manifest spherical equivalent refraction; **UDVA** = uncorrected distance visual acuity; **UV-A** = ultraviolet A.

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