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Corneal crosslinking in keratoconus management.

Le crosslinking de la cornée dans la prise en charge du kératocône.

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Mots Clés: cornée; kératocône; crosslinking; **Key words:** cornea; keratoconus; crosslinking

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1 **Corneal crosslinking in keratoconus management.**

2 **Le crosslinking de la cornée dans la prise en charge du k ratoc ne.**

3

4 **Abstract**

5 Since two decades, corneal crosslinking (CXL) has been proposed as the sole therapeutic
6 option to halt progression of keratoconus or other ectatic diseases. CXL aims at stiffening the
7 cornea using a combination of ultraviolet-A light and a chromophore (vitamin B2, riboflavin),
8 and has been proposed in various indications, from progressive ectatic diseases to corneal
9 infection. Despite being in clinical use for many years, many controversies and discrepancies
10 exist towards CXL procedure and its exact role is still under debate. We report an up-to-date
11 review of the state of the art of CXL and describe the basic principles, the different existing
12 CXL techniques reporting basic and clinical evidence, as well as the new perspectives and the
13 possible future developments of the procedure.

14 Keywords : cornea; keratoconus; crosslinking

15

16 **R sum **

17 Depuis 2 d cennies, le crosslinking de la corn e (CXL) s'est impos  comme la seule
18 alternative th rapeutique possible pour contrecarrer la progression du k ratoc ne ou d'autres
19 maladies ectatiques corn ennes. Le CXL, traitement visant   solidifier la corn e   l'aide d'un
20 chromophore (la riboflavine, ou vitamine B2) irradi  par une lumi re  mise dans le spectre
21 des ultra-violets, a  t  propos  dans de nombreuses indications, allant des ectasies corn ennes
22 progressives aux abc s de corn e. Malgr  son utilisation courante en pratique clinique depuis
23 de nombreuses ann es, de multiples controverses et contradictions existent   ce jour et son
24 r le exact demeure discut . Nous reportons ici une revue de la litt rature se voulant  tre une
25 mise   jour des connaissances actuelles concernant le CXL en d crivant ses grands principes

26 biologiques et pratiques, ainsi que les données scientifiques cliniques existantes et ses
27 possibles évolutions futures.

28 Mots-clefs :cornée ; kératocône ; crosslinking

29

30 **Introduction**

31 Keratoconus, the most common primary ectatic disorder, is a noninflammatory corneal
32 deformation with progressive, inferior corneal thinning, steepening, and late apical scarring
33 [1]. Keratoconus generally starts during the second decade of life with a variable progression
34 that tends to naturally stop around the third decade. It affects all ethnic groups and both
35 genders although it is seen more commonly in countries of the Mediterranean. Although the
36 exact physiopathology of keratoconus progression is still unknown to date, both
37 environmental and genetic factors have been incriminated and frequent vigorous eye rubbing
38 is a well-established risk factor of progression. Other primary forms of corneal ectasia include
39 pellucid marginal degeneration and keratoglobus, while secondary forms are mainly related to
40 corneal refractive surgery: Laser Assisted In Situ Keratomileusis (LASIK), Small Incision
41 Lenticule Extraction (SMILE) or Photorefractive Keratectomy (PRK) procedures [2].

42 Keratoconus treatments aim at overcoming refractive limitations with optimal refractive
43 correction with glasses or rigid-gas permeable contact lenses (RGPCl) or intracorneal ring
44 segments (IRCS) implantation, stabilizing the disease in case of progression and restoring
45 corneal transparency with corneal transplantation in case of severe visual impairment due to
46 apical scarring of corneal hydrops. Since visual outcomes following penetrating keratoplasty
47 (PK) or deep anterior lamellar keratoplasty (DALK) are usually limited for various reasons
48 (i.e. giant astigmatism, adverse events during surgery or follow-up, graft decompensation),
49 avoiding or postponing surgery by halting keratoconus progression is crucial.

50 Corneal crosslinking (CXL) with riboflavin and UVA is a treatment modality for keratoconus
51 that was first developed in Dresden, Germany in 1998 [3,4] and described in a clinical
52 practice by Wollensak in 2003 [5]. CXL has been proposed as the first existing treatment to
53 aim at strengthening corneal tissue [6] and halting progression of keratoconus or secondary
54 corneal ectasia. However, no existing treatment aims at impeding the exact underlying

55 physiopathology of the keratoconus as this latter point remains poorly known to date. We
56 report an up-to-date review of the state of the art of CXL and describe the basic principles, the
57 different existing CXL techniques reporting basic and clinical evidence, as well as the new
58 perspectives and the possible future developments of the procedure.

59

60 **Biological principles of corneal CXL**

61 *1. Physicochemical basis*

62 Collagen is a triple helical structural protein present abundantly in the corneal extracellular
63 matrix. Corneal CXL is based on a photochemical reaction between a photo-sensitizer and a
64 specific light irradiation that induces intermolecular covalent bonds “cross-links” between the
65 amino-terminals of the collagen side chains and the proteoglycans of the extracellular matrix
66 [7]. Riboflavin (vitamin B2), the standard photo-sensitizer used in corneal CXL, is excited
67 when irradiated by a light beam of a specific wavelength (absorption peak: UV-A range),
68 inducing modifications to the corneal structure and increasing corneal stiffness [8].

69 Although induction of crosslinks is a well-established procedure in polymer chemistry to
70 increase the elastic modulus of materials, the photochemically induced effect of CXL in the
71 cornea cannot be evidenced directly by staining methods or microscopic techniques. Instead,
72 CXL effect on corneal tissue is deduced from several indirect signs and changes to collagen-
73 containing tissue [9]. Stress-strain measurements performed in *ex-vivo* studies on human and
74 porcine corneas documented an increased corneal rigidity after CXL, that is correlated to the
75 collagen concentration rate and the age [8,10], explaining the propensity to keratoconus
76 stabilization with age. Moreover, it has been reported that porcine crosslinked corneas showed
77 a reduced tendency to swelling and hydration when compared to untreated controls [11]. *Ex-*
78 *vivo* studies on corneas showed an increase of collagen fibers thickness after CXL treatment
79 [12] and modifications in the collagen fibers diameter, interfibrillar spacing, and

80 proteoglycans area, resulting in a more uniform distribution of collagen fibrils, a key feature
81 for corneal transparency [13]. In an *ex-vivo* study, Wollensak and colleagues [8] reported a
82 nearly 3.5 fold increase in Young's modulus of human corneas after CXL. To date, most of
83 *ex-vivo* studies confirmed the efficiency of CXL in increasing the corneal strength. CXL
84 procedure also improves the corneal resistance to degradation processes and enzymatic
85 digestion mediated by pepsin, trypsin and collagenase with lengthening of the turnover time
86 of the collagen [14].

87 2. *The role of oxygen*

88 Oxygen plays an important role during CXL procedure [15], impacting efficiency and corneal
89 stiffening. The CXL photoreaction is driven by the interaction of corneal stromal oxygen,
90 riboflavin, and UVA irradiation and can follow two different reaction pathways depending on
91 local oxygen availability. The type I reaction pathway occurs in low-oxygen conditions where
92 toxic hydrogen peroxide is generated as a final product. In the aerobic (nonhypoxic) type II
93 reaction, the riboflavin triplets react directly with oxygen to form the less toxic singlet oxygen
94 that cross-links collagen. Hence, riboflavin stromal impregnation and irradiation time and
95 energy are not the only variables that must be taken into account in the equation. Maintaining
96 aerobic conditions during the CXL procedure is essential to limit type I mechanism-related
97 toxic reactive oxygen species formation.

98 3. *Corneal biomechanics*

99 The cornea exhibits complex viscoelastic properties, and evaluating its biomechanical
100 properties is challenging as the tissue response depends on the strain rate. CXL targets the
101 anterior 40% of the corneal stroma which is the strongest region according to tensile strength
102 studies in human donor corneas [16].

103 The ORA [17] (Ocular Response Analyzer; Reichert Ophthalmic Instruments, Buffalo, NY), a
104 non-contact tonometer (NCT) was the first machine able to measure *in-vivo* corneal

105 biomechanics. Although ORA measurements are significantly lower in keratoconus when
106 compared to healthy corneas, [18,19] most of clinical studies have failed to show any
107 improvement in corneal hysteresis (CH) and corneal resistance factor (CRF) after CXL to
108 date, probably because of the high variability of the ORA and the low statistical power of
109 most of the existing studies [20–24].

110 The Corvis ST (Oculus, Wetzlar, Germany), a more recent NCT associated with a
111 Scheimpflug camera, provides more detailed evaluation of corneal biomechanics than ORA.
112 Some authors reported improvements in various Corvis ST measurements after CXL
113 treatment [25,26].

114 Corneal biomechanics is a subject of tremendous interest for clinical research and many other
115 approaches have been proposed to assess *in-vivo* corneal biomechanical changes after CXL,
116 but are not readily available in daily clinical practice to date like applanation resonance
117 tonometer or ART [27], corneal elastography [28] or Brillouin optical microscopy [29–31].

118 4. *In-vivo aspects following CXL*

119 4.1. *In-vivo confocal microscopy (IVCM)*

120 Histological studies suggested that the morphologic and optical changes associated with CXL
121 are typically restricted to a depth of 200 to 300 μm from the anterior surface [8,32]. These
122 findings are consistent with similar results with IVCM examinations of crosslinked corneas
123 revealed distortion of the basal epithelium, typical of keratoconus, a significant decrease in
124 keratocytes density and constant and early obliteration of the subbasal plexus nerve with
125 neuroregeneration during the first months. Nerve and keratocytes regeneration over time was
126 faster following iontophoresis CXL (I-CXL) than epithelium-off techniques as at 6 months
127 post-operatively, full cellular regeneration is obtained in I-CXL versus 50% of regeneration in
128 conventional CXL (C-CXL) or accelerated CXL (A-CXL) [33]. After CXL procedure,
129 corneal nerves appear more distorted (**Figure 1**) and exhibit a fragmented appearance before

130 forming an interconnected network [33] as the ocular surface sensitivity decreases in the first
131 months postoperatively with progressive recover to normal preoperative levels within 1 year.
132 After the first year, tear production measured by the Schirmer test and the tear break-up time
133 is within normal range [34].

134 Abnormal stromal microstriae that appeared as multiple, thin, dark lines in contrast with the
135 brighter reflectivity of the stroma are also visible. Loss of keratocytes with honeycomb edema
136 and apoptotic bodies is observed till 3 months and keratocytes regeneration is complete
137 between 6 to 12 months [35]. The endothelium is usually not affected by the CXL procedure.
138 Interestingly, the exact mechanisms behind corneal CXL responsible for corneal stiffness
139 improvement remain poorly known to date. In 2018, a comparative study showed that PRK
140 was safe in keratoconus, and one could suppose that the induced corneal haze could
141 participate to stabilize corneal ectasia [36]. Furthermore the alteration of the subbasal plexus
142 nerve after CXL might imply a decrease in ocular pruritus and therefore decreasing eye
143 rubbing. Plus, CXL reduces stromal melting susceptibility. Hence, CXL might halt
144 keratoconus progression via various mechanisms: fibrosis, collagen cross-links induction,
145 stromal melting diminution and pruritus sensation inhibition.

146 4.2. *Anterior segment OCT (AS-OCT)*

147 AS-OCT plays a major role in keratoconus healthcare both at early and advanced stages. An
148 AS-OCT based classification has recently been proposed to grade keratoconus severity [37].
149 Recently, AS-OCT has been shown to improve the diagnostic sensitivity for keratoconus
150 progression [38] as well as the detection of forme fruste keratoconus (FFKC) by intelligence
151 analysis of the Bowman's layer [39]. CXL aspect on AS-OCT exhibits typical signs, the most
152 relevant being the variable existence of a hyperreflective stromal line or demarcation line
153 (DL)] of various depth depending on the CXL protocol, most visible during the first month
154 postoperatively that tends to fade with time. Although some authors suggested that the depth

155 of the DL marks the border of the corneal stroma that is denuded from keratocytes [40] and
156 represents an indicator of the effectiveness of CXL treatment as it is deeper and more visible
157 in C-CXL versus A-CXL and epi-on techniques (**Figure 2**), most of recent studies argue
158 against this suggestion suggesting that if even the presence of a DL is related to CXL
159 effectiveness, a higher DL depth is not correlated with higher CXL efficacy nor with the
160 probability of lower failure [41].

161 Numerous CXL protocols exist to date, and the microstructural corneal alteration differences
162 found in studying *in-vivo* imaging following CXL procedures highlight probable differences
163 in corneal biomechanics improvement following each approach. Hence, *in-vivo* corneal
164 imaging could possibly help to understand the underlying mechanisms of CXL and to
165 determine predictive factors for CXL failure.

166

167 **CXL protocols in keratoconus**

168 *1. Conventional CXL or Dresden protocol (C-CXL)*

169 The conventional CXL (C-CXL) or Dresden protocol is the first CXL technique described in
170 the literature (Wollensak and colleagues, 2003 [5]) and is currently considered as the “gold
171 standard” treatment. Since the epithelial tight junctions block riboflavin absorption, C-CXL
172 requires the mechanical or excimer laser-guided epithelium debridement in the central zone of
173 the cornea under sterile condition. Riboflavin is then applied to the corneal surface during 30
174 minutes to obtain sufficient stromal impregnation and followed by 30 minutes continuous
175 application of ultraviolet (UV-A, wavelength 370 nm) irradiation at an irradiance of 3.0
176 mW/cm², resulting in a total surface dose of 5.4 J/cm². This protocol, suffers for 2 major
177 pitfalls. First, epithelial debridement is mandatory to facilitate penetration of riboflavin into
178 the cornea and to obtain sufficient penetration of the Riboflavin into the stromal bed. Second,
179 the C-CXL protocol is time consuming for both surgeon and patient. To overcome these two

180 pitfalls, various alternative protocols have been proposed that retain the corneal epithelium or
181 to reduce the surgery time.

182 2. *Accelerated CXL (A-CXL)*

183 The Bunsen-Roscoe law (BRL) of reciprocity states that a photochemical reaction is directly
184 proportional to the total energy dose irrespective of the administered regime. Consequently,
185 after Wollensak first publication in 2003, several accelerated protocols with application of
186 higher irradiances over shorter times have been proposed as an equivalent alternative to the
187 Dresden protocol in an effort to reduce treatment times. Kanellopoulos and colleagues [42]
188 reported the first randomized prospective contralateral eye study on A-CXL (7mW/cm² for 15
189 minutes) showing similar clinical results in ectasia stabilization when compared to the
190 Dresden protocol. A-CXL protocols are in constant evolution, especially concerning the
191 relationship between treatment time and UV intensity. Any protocol (i.e. 9 mW/cm² for 10
192 minutes, 18 mW/cm² for 5 minutes or 30 mW/cm² for 3 minutes [43]) that delivers a surface
193 dose of 5.4 J/cm² could be considered comparable to the Dresden protocol. However, A-CXL
194 protocols yield variable results, as experimental and clinical reports demonstrated significant
195 differences between the two treatment approaches concerning the demarcation line depth [44]
196 and the efficacy as described by the induced corneal stiffness [43]. In 2018, a meta-analysis
197 [45] concluded that C-CXL is more effective but A-CXL may be less likely to reduce central
198 corneal thickness (CCT) and corneal endothelial cell loss. A drop in efficacy is observed
199 when higher irradiances are used [46]. Several hypothesis have been suggested to explain
200 these discrepancies, with the most prominent being higher oxygen depletion rate in A-CXL,
201 especially in shortest irradiation time protocols, reducing the efficacy of the photochemical
202 reaction [47]. As a result, pulsed and oxygen enriched CXL were introduced in an effort to
203 replenish oxygen in the cornea during high fluence treatments.

204 3. *Oxygen Enriched High Intensity CXL*

205 Oxygen plays an important role during CXL procedure, impacting efficiency and corneal
206 stiffening. CXL beneficial effect on the cornea occurs under aerobic conditions (type II
207 mechanism). After the first 10–15 seconds of the reaction, oxygen becomes totally depleted,
208 and toxic oxygen-free radicals are generated by riboflavin photolysis (type I mechanism)
209 explaining the drop of CXL efficacy of A-CXL procedures when higher fluences are used as
210 oxygen is rapidly depleted and its stromal rediffusion is prevented. In an attempt to increase
211 CXL effect on corneal biomechanics, some authors suggested to optimize epithelium-on or
212 epithelium-off accelerated CXL protocols with oxygen enrichment [48–50].

213 4. *Pulsed CXL (p-CXL)*

214 Similarly to oxygen enriched high intensity CXL, pulsing UVA radiation (i.e. 1 second on – 1
215 second off) have been proposed to achieve higher oxygen concentrations by reducing oxygen
216 consumption and to enhance the efficacy of CXL treatment. Pulsed CXL (p-CXL) has been
217 described in both epi-off (p-ACXL) and epi-on techniques (p-ICXL) with interesting results
218 [51].

219 5. *Transepithelial CXL (epithelium-on techniques)*

220 5.1. *The epithelial barrier*

221 Epithelial debridement is responsible for post-operative ocular pain and most of the severe
222 adverse effects of CXL (sterile infiltrates, infectious keratitis, and persistent epithelial defect).
223 Therefore, retaining the corneal epithelium would dramatically increase the safety of CXL.
224 Riboflavin phosphate is a negatively charged hydrophilic molecule and its permeability into
225 the corneal epithelium is naturally limited due to the lipophilic epithelial membrane and the
226 strong repulsion by corneal proteoglycans [52]. Consequently, although its molecular weight
227 is low, riboflavin penetration through intact epithelium is a real challenge and standard

228 formulations used in epi-off techniques do not reach the desired intrastromal concentration
229 through an intact epithelium [53].

230 *5.2. Transepithelial CXL (TE-CXL)*

231 First strategies for performing effective TE-CXL used riboflavin formulations associated with
232 “permeation enhancers” (such as alcohol [54], benzalkonium chloride [55] or other
233 epitheliotoxic molecules [56,57]) designed to overcome epithelial permeability barriers. These
234 early TE-CXL exhibited poor outcomes in clinical trials and might lead to focal epithelial
235 damage due to the drugs toxicity. Because of the convincing evidence of insufficient efficacy
236 of TE-CXL protocols, these approaches are rarely used in clinical practice [57–59].

237 *5.3. Iontophoresis CXL (I-CXL)*

238 Iontophoresis was developed to ensure sufficient ionized riboflavin penetration through the
239 intact epithelium by applying a small electric current. Transepithelial iontophoretic riboflavin
240 loading demonstrated corneal stromal penetration similar to the standard CXL protocol [60]
241 and numerous studies demonstrated promising results using I-CXL with cessation of
242 keratoconus progression with improvements in visual acuity and topographic outcomes has
243 been documented both in adults and pediatric patients [33,61,62]. However, long-term studies
244 seem to highlight a higher failure rate than C-CXL [63,64], from 1.3% to 27.8% depending on
245 the initial keratoconus severity (**Table 1**). There have also been rapid, ongoing developments
246 in order to enhance I-CXL efficacy such as the pulsed-light enhanced-fluence I-CXL protocol
247 (EF I-CXL), yielding promising results [51].

248 *5.4. Epithelial-disruption collagen crosslinking (ED-CXL)*

249 Another approach [65] was to cause mechanical disruption of the epithelium with a corneal
250 disruptor device, in order to permit the diffusion of riboflavin into the stroma. ED-CXL
251 approach remains anecdotal as the epithelial disruption is equivalent to a partial debridement

252 and this technique has the same safety risks associated with standard CXL as it broaches the
253 epithelial integrity.

254 *5.5. Stromal “Pocket” Corneal CXL*

255 In 2009, Kanellopoulos [66] described a novel and rapid soak-and-treat method where
256 riboflavin solution is injected into a 100- μ m deep intrastromal pocket incision created by a
257 femtosecond laser in early keratoconus. In 2014, another study [67] in porcine eyes reported
258 that the biomechanical effect was 50% less pronounced than that after C-CXL. No other
259 studies have been reported to date concerning this approach.

260 *5.6. Future perspectives of epithelium-on CXL*

261 Further developments towards TE-CXL concern the design of new riboflavin formulations
262 that do not alter the integrity of the epithelium. Various molecules designed to enhance
263 epithelial permeability by the addition of epithelial-toxic agents as MedioCross TE (Peschke
264 Meditrade GmbH, Waldshut-Tiengen, Germany, formulation: 0.25% Riboflavin,
265 Hypromellose (HPMC), Benzalkonium Chloride), Paracel plus (Avedro, Inc., formulation:
266 0.25% Riboflavin, HPMC, sodium edetate (EDTA), trometamol, benzalkonium chloride,
267 saline - supplemental oxygen), VibeX Xtra (Avedro, Inc., formulation: isotonic solution of
268 0.25 Riboflavin, saline), Ribocross TE (IROS Srl, Napoli, Italy, formulation: 0.125%
269 Riboflavin, D-alpha-tocopheryl polyethylene glycol, succinate - vitamin E solution) and
270 Ribostat CXLO (CXLO, CXL Ophthalmics LLC, dextran-free formulation: Riboflavin +
271 sodium iodide) have been used in several studies [68,69]. In a prospective, nonrandomized
272 clinical trial of 25 eyes with a 2-year follow-up, Ribocross TE (a vitamin E-enhanced
273 riboflavin formulation) demonstrated statistically significant improvements in visual acuity,
274 refraction, and corneal topography [70]. In a prospective observational study, Stulting and
275 colleagues [71] reported stable improvement of outcomes on 512 eyes treated with pulsed TE-
276 CXL (4 mW/cm², 30 minutes) using Ribostat CXLO dextran-free formulation over 2 years,

277 inherently safer than epi-off CXL, reducing the risk of infectious keratitis and delayed
278 epithelial healing, allowing bilateral simultaneous treatment.

279 Development of novel devices or formulations to deliver proper concentration of
280 photosensitizer into the stroma is of key importance. Nanoparticles are a promising agent
281 acting as a vector to allow a molecule to penetrate into epithelium cells without altering the
282 epithelium integrity. In an *ex-vivo* study on rabbit corneas, Bottos and colleagues [72] found
283 similar absorption of a biocompatible riboflavin-based amphiphilic nanoemulsion through an
284 intact epithelium than de-epithelialized corneas. In 2017, Lombardo and colleagues [73]
285 showed that a nanotechnology-based riboflavin 0.1% solution was effective in enriching the
286 anterior stroma with riboflavin through the intact epithelium of human donor corneas. Labate
287 and colleagues [74] showed that TE-CXL using a modified Riboflavin with polymeric
288 nanoparticles of cyclodextrin was effective to improve the biomechanical strength of donor
289 human corneas.

290 6. Customized Topography-Guided CXL (TG-CXL)

291 TG-CXL was developed in 2011 [75] as a customized treatment centered on the ectatic cone
292 aiming at delivering more energy on the weakest parts of the cornea and limiting the toxicity.
293 Indeed, a smaller, focal treatment centered on the topographic cone might provide enhanced
294 flattening effects and reduce the high order aberrations. Therefore, TG-CXL was initially
295 proposed as a customize approach to improve optical predictability of CXL and maximizing
296 corneal regularization. Furthermore, a smaller treatment zone and a smaller epithelial defect
297 might also limit the CXL toxicity with less corneal haze, less UV exposure of the corneal
298 endothelium, limbal stem cells, and intraocular tissues. In a prospective, nonrandomized
299 clinical trial, Cassagne and colleagues [76] showed that TG-CXL seems to be as safe as C-
300 CXL with stronger flattening in Kmax and better improvement in CDVA at 1 year
301 postoperatively.

302 7. *Future perspectives*

303 CXL technique remains in constant evolution and various ongoing developments are still
304 under evaluation to improve CXL efficiency and reduce its disadvantages. In attempts to
305 reduce C-CXL drawbacks, variations on time surgery and epithelium-on techniques also
306 reduce oxygen diffusion or Riboflavin penetration resulting in less efficacy of the CXL.
307 Recently, some authors suggested that CXL procedure could be performed at the slit lamp
308 rather than in the operating room in order to reduce the procedure time and related costs [77].
309 The ideal CXL procedure would significantly increase the corneal strength to halt keratoconus
310 progression with minimal cellular toxicity and minimal time of surgery with no need of
311 epithelial debridement.

312 Other photo-inducers have been evaluated as an alternative to riboflavin as Rose Bengal (RB)
313 plus green light (RGX) [78]. RB penetrates approximately 100 μm into the corneal stroma in
314 rabbit eyes, where it absorbs the incident green light. RGX has been shown to increase the
315 elastic modulus selectively in the anterior stroma and the corneal stiffness in a shorter surgery
316 time than C-CXL (12 minutes total time) with minimal keratocyte toxicity [79]. Similarly,
317 other chromophores have been proposed such as eosin (green light at 532 nm) or WST-D, a
318 watersoluble bacteriochlorophyll-derived photosensitizer molecule already used as an
319 intravenous treatment for prostate cancer (near infrared light at 755 nm) [80].

320 Other methods of purely chemical CXL were also investigated such as decorin [81], a small
321 naturally occurring proteoglycan can bridge collagen fibrils, aliphatic beta-nitroalcohols [82]
322 or genipin [83], an active molecule derived from the plant *Gardenia jasminoides*. These have
323 potential advantages as avoidance of radiation toxicity and epithelial removal and a more
324 simple delivery method of the active agent.

325 **CXL in thin corneas**

326 Based on *in-vitro* studies [84], an endothelial cytotoxic effect is expected when pachymetry
327 after de-epithelialization is below 400 μm (threshold: 0.35 mW/cm^2). As keratoconus severity
328 is correlated with corneal thickness, and patients with thin corneas are those who would
329 benefit the most from CXL treatment. Along with TE-CXL and I-CXL that do not require
330 epithelial debridement, several alternative techniques have been suggested to treat thin
331 corneas that would otherwise not be eligible for treatment.

332 *1. Hypo-osmolar Riboflavin*

333 In 2009, Hafezi and colleagues [85] described a modified C-CXL protocol using hypotonic
334 riboflavin without dextran (310 mOsm/L instead of 402.7 mOsmol/L for standard riboflavin
335 in 20% dextran) to temporarily induce corneal swelling in thin corneas. To avoid corneal
336 desiccation during the irradiation phase resulting in decreasing in pachymetry, a modified A-
337 CXL using 9 mW/cm^2 for 10 minutes should be preferred [86,87].

338 *2. Contact lens-assisted Crosslinking (CA-CXL)*

339 Jacob and colleagues [88] described C-CXL technique for thin corneas (pachymetry of 350
340 μm after debridement) using a bandage contact lens during the procedure to artificially
341 increase corneal thickness. They found this technique to be effective in their study of 14 eyes.
342 The advantage of this technique is that it is not dependent on the swelling properties of the
343 cornea. On the other hand, the riboflavin-soaked contact lens reduces oxygen availability and
344 absorbs UVA radiation [89].

345 *3. Lenticule-assisted Crosslinking*

346 Similarly to CA-CXL, Sachdev and colleagues [90] described a modified C-CXL technique
347 after placing stromal lenticule removed from SMILE surgeries and placed over the apex of the
348 cone to artificially increase corneal thickness. Thereby, the thickness of the lenticule can be

349 customized and is also biologically similar to the treated cornea. This technique remains
350 anecdotal.

351

352 **Clinical application in progressive keratoconus**

353 Defining disease progression and the threshold for surgical intervention in keratoconus
354 patients under observation remains a challenge. Due to lower repeatability of AS-OCT and
355 topographic imaging in keratoconus versus normal corneas [91], a change in Kmax of +1.5D
356 vs baseline on topographic image comparison maps is an interesting threshold for CXL
357 treatment [92]. However, a lower threshold (+1D vs baseline Kmax) is used in most of
358 contemporary studies [93–95]. Changes in other criteria (**Table 2**) may refine keratoconus
359 progression assessment when the observed increase in Kmax is below +1.5D. When possible,
360 rigid contact lenses should be left out for a minimum of 2 weeks before each topography
361 examination to reduce corneal warpage. AS-OCT imaging may also help to refine more
362 precisely progression of keratoconus assessment as epithelial thickness profile measurements
363 have been proposed as an adjunctive follow-up tool for monitoring the efficacy of the
364 treatment [96]. **Table 1** presents the CXL efficacy data for adult keratoconus. Recent and
365 relevant articles published from 2015 onwards about CXL in adult keratoconus with a
366 reported failure rate were selected and are listed by chronological order.

367

368 **Pediatric keratoconus**

369 Pediatric cases often present with keratoconus that progress more rapidly than adult onset
370 keratoconus. Chatzis and colleagues [97] showed that 88% of pediatric cases progress over a
371 short period of time and some authors have suggested that CXL in children and adolescents
372 should be performed as soon as the diagnosis has been made without any documentation of
373 progression [98]. Other authors recommended a close monitoring of pediatric cases with more

374 frequent follow-up visits than adults (i.e. every 1-3 months instead of 6-12 months in adults)
375 and CXL treatment as soon as progression is documented [99]. Clinicians must keep in mind
376 that keratoconus is usually more aggressive in children than in adults and control of chronic
377 habits of abnormal eye rubbing is usually uncertain in the pediatric context. Although C-CXL
378 has proven its efficacy through long-term studies [100] and constitutes the gold standard
379 protocol in aggressive cases, this procedure can be difficult to perform in the pediatric
380 context, and might require general anesthesia in selective cases. In less aggressive
381 keratoconus, A-CXL could be preferred to improve patient compliance during the procedure.
382 Eissa and colleagues [101] reported that A-CXL and C-CXL were effective in a prospective,
383 randomized contralateral eye comparative study in 68 pediatric keratoconus eyes. Invasive
384 approaches such as epithelium-off CXL, need to be weighed against the risks and benefits,
385 especially in the pediatric care context. Indeed, potential sight-threatening serious CXL-
386 related adverse effects (corneal haze, infectious keratitis...) could be particularly devastating
387 in children. In less aggressive cases and when advice to avoid eye rubbing is well understood
388 by the patient, TE-CXL protocols such as I-CXL can be performed. **Table 3** presents the CXL
389 efficacy data for pediatric keratoconus. Recent and relevant articles published from 2015
390 onwards about CXL in pediatric keratoconus with a reported failure rate were selected and are
391 listed by chronological order.

392

393 ***Beyond the controversies***

394 To date, most of clinical studies have failed to show any improvement in corneal *in-vivo*
395 biomechanical properties [20–24]. Although *ex-vivo* studies have documented an increased
396 corneal rigidity after CXL [8,10], it remains unclear whether corneal CXL truly improve *in-*
397 *vivo* corneal strength or not. Molecular bonds between collagen bundles that are supposed to
398 be induced by CXL procedure cannot be evidenced directly [9] and one could assume that

399 CXL might halt keratoconus progression via various mechanisms that are not necessarily
400 correlated to the initial expected effect. Corneal CXL induces corneal nerve modification,
401 keratocytes apoptosis and fibrosis and corneal haze that are visible in IVCM within the first
402 months postoperatively [33]. The alteration of the subbasal plexus nerve after CXL might
403 help to decrease ocular pruritus sensation. In 2018, a comparative study showed that PRK was
404 safe in keratoconus, and one could suppose that the induced corneal haze could also
405 participate to stabilize corneal ectasia [36]. CXL procedure also reduces stromal melting
406 susceptibility by improving the corneal resistance to degradation processes and enzymatic
407 digestion mediated by pepsin, trypsin and collagenase with lengthening of the turnover time
408 of the collagen [14]. Hence, CXL could halt keratoconus progression via various mechanisms:
409 fibrosis or corneal haze induction, collagen cross-links induction, stromal melting and pruritus
410 sensation inhibition. Furthermore, various biases might hinder keratoconus documentation
411 after CXL. Both epithelium-off and epithelium-on techniques induce epithelial remodeling
412 [96] which might alter the evaluation of the anterior corneal curvature map and interfere in
413 keratoconus progression assessment (**Figure 3**). Hence, most of the existing controversies
414 result from two major issues: the uncertainty about CXL true effectiveness in stiffening the
415 cornea *in-vivo* and the poor understanding of the exact mechanisms of CXL in halting
416 keratoconus progression.

417 In 2008, Dresden group reported results of a retrospective study [102] of 241 eyes with a
418 follow-up of 6 years showing significant decrease in keratometric values, astigmatism and
419 improvement of visual acuity after C-CXL, with high stability and safety of this procedure
420 demonstrated in another 10-year-follow-up study [103]. A major meta-analysis [104]
421 including randomized controlled trials (RCT) and prospective studies concluded that if the
422 effectiveness of CXL in halting the progression of keratoconus seems real, numerous
423 limitations exist including the high level of heterogeneity between the studies, the poor

424 reporting of drop-out rates and loss to follow-up and the lack of control studies with
425 comparative matched cohort as well as well-conducted long-term studies. In fact, the majority
426 of the existing studies concerning CXL consist of case series that cannot provide evidence of
427 causal effect. A meta-analysis on 6 RCT evaluating C-CXL [105] showed statistically
428 significant improvement in the maximum keratometry reading (mean: -2.05 D), minimum
429 keratometry reading, mean keratometry reading, visual acuity and manifest cylinder error
430 versus control. These results are concordant with many other studies [5,106,107]. Two meta-
431 analysis on 23 heterogeneous studies with 1557 eyes [108] and 5 RCT with 289 eyes [109]
432 concluded that CXL might effectively halt the progression of keratoconus, although evidence
433 is limited due to the significant heterogeneity and methodological weaknesses of the
434 individual studies. Another meta-analysis on 22 studies with 1158 eyes [110] comparing
435 mixed A-CXL protocols versus C-CXL for adult progressive keratoconus (C-CXL: 577 eyes;
436 A-CXL: 581 eyes) concluded that if both protocols provided successful results, C-CXL was
437 superior to A-CXL concerning the DL depth and improvement in Kmin values, that might
438 indicate higher treatment efficacy.

439 Despite these controversies, the majority of the panelists for the Global Delphi Panel of
440 Keratoconus and Ectatic Diseases [111] for keratoconus declared performing CXL in clinical
441 practice for progressive keratoconus as majority of cornea specialists suppose a beneficial
442 effect of CXL on corneal biomechanics.

443

444 **CXL failure, safety and adverse effects**

445 Treatment failure that occurs in 8.1–33.3 % of the cases according to a 2015 review [112] on
446 51 heterogeneous articles on epi-off and epi-on techniques is defined in most of the existing
447 studies as continued progression with an increase in maximum K readings of 1.0 D over the
448 preoperative value. A high preoperative Kmax-value and a young age were negative

449 predictors for CXL failure in many studies. No strong evidence is available on the benefit to
450 repeat CXL in case of treatment failure. Indeed, in an *in-vivo* study on mice corneas, Tabibian
451 and colleagues [113] showed that repeated CXL 3 days after the first procedure did not
452 improve corneal stiffness. Similarly, another *ex- vivo* study [114] on human corneas suggested
453 that no further cross-links are induced when CXL treatment is immediately repeated.
454 However, in 2015, Antoun and colleagues [115] showed that repeated C-CXL was effective in
455 halting progression in 7 eyes. In 2020, Turhan and colleagues [116] showed that repeated epi-
456 off A-CXL (9 mW/cm²) was effective in halting keratoconus progression in 12 eyes with
457 documented progression despite a first epi-off or epi-on CXL procedure. Mean interval
458 between the two procedures was of 19 months.

459 The most common complications reported after the procedure are pain, stromal haze and
460 sterile infiltrates [117]. More serious but rare events include infection, corneal melting,
461 perforation and ulceration, and stromal scarring. Infectious keratitis or corneal melting and
462 perforation have been reported in many case reports but remains rare adverse events. A recent
463 prospective cohort study [118] on 670 eyes reported a post-operative infectious rate of 1.6%.

464

465 **Combined procedures: CXL-Plus**

466 *1. Combined CXL and photorefractive keratectomy*

467 The key objective of this treatment involves halting the progression of ectasia and improving
468 the refractive errors simultaneously. Options for epithelium debridement for cross-linking
469 include mechanical debridement or excimer laser phototherapeutic keratectomy (PTK). As
470 regional epithelial thickness is highly variable in keratoconus [119], AS-OCT epithelial
471 measurements and transepithelial PTK may help to normalize the cornea, and resulted in
472 better visual and refractive outcomes than mechanical debridement in a prospective
473 comparative study [120]. Surface ablation can also be customized using topoguided-PRK

474 customized software (TG-PRK). There is uncertainty on the optimal timing of these
475 procedures, and whether they should be conducted sequentially or simultaneously. Kymionis
476 and colleagues reported interesting results following simultaneous TG-PRK with CXL for
477 keratoconus [121] and PMD [122]. Similarly, Kanellopoulos and colleagues [123] suggested
478 that simultaneous treatment was superior to sequential treatment with better visual outcomes,
479 spherical equivalent refraction, and change in keratometry as well as higher safety with more
480 corneal haze in the sequential approach. There is no consensus on the maximum stromal
481 ablation depth or the use of mitomycin C to date, but most of authors recommend to be as
482 conservative as possible [124]. **Figure 4** illustrates a clinical case of a patient treated with
483 CXL plus TG-PTK.

484 2. *CXL-ICRS*

485 ICRS implantation improves visual and topographic outcomes in patients with keratoconus
486 but do not prevent disease progression, [125] so combination treatment with crosslinking may
487 be more beneficial than either treatment alone. Although the combination of CXL with ICRS
488 implantation may be an interesting approach as riboflavin can be directly introduced into the
489 stromal bed through the ring segment channels created by modified femtosecond laser
490 settings, iatrogenicity is increased as corneal haze is more frequent and refractive
491 predictability is lowered [126]. In this combined approach, ICRS implantation is generally
492 performed prior to the CXL procedure to minimize the femtosecond laser energy delivered to
493 the cornea, and both simultaneous or sequential combined CXL-ICRS approach have shown
494 improvement in visual acuity and topography in keratoconus and ectasia [127,128]. In
495 conclusion, CXL-Plus should be discussed as a treatment option with those keratoconus
496 patients who wish to improve their uncorrected and corrected visual acuity. In a prospective
497 non randomized interventional study on 452 eyes, Singal and colleagues [129] showed that
498 implantation of ICRS induces corneal flattening of both the anterior and posterior surfaces,

499 which contribute to the decrease in keratometric power and greater improvement in
500 astigmatism and corrected distance visual acuity (CDVA). Hence, CXL-ICRS is preferable
501 for eyes with more irregular astigmatism and worse corrected CDVA and CXL-TG-PRK for
502 eyes requiring improvements in irregular astigmatism but still have good CDVA. **Figure 5**
503 illustrates a clinical case of a patient treated with CXL plus TG-PTK and ICRS implantation.

504

505 **Other clinical applications of CXL**

506 *1. CXL in other ectatic diseases*

507 In 2017, a meta-analysis [130] showed that CXL is a promising treatment to stabilize
508 iatrogenic keratectasia after LASIK or PRK surgery, with significant improvement in visual
509 acuity. Actually there is no distinct difference in the management of progressive keratoconus,
510 pellucid marginal degeneration (PMD) or iatrogenic keratectasia in literature, and the use of
511 CXL to stabilize the cornea and stop progression in these particular cases is generally the rule.
512 Plus, no distinct definition of parameters of progression specific for PMD or iatrogenic
513 keratectasia exists to date.

514 *2. Prophylactic CXL in refractive surgery: LASIK-Xtra, PRK-Xtra, SMILE-Xtra*

515 Several authors suggested the utility of CXL as an adjuvant treatment of eyes with high-risk
516 of post-operative ectasia following refractive surgery. For prophylactic use, virtually any
517 patient could be treated with CXL to reduce the chance of future development of ectasia,
518 especially patients with thinner than normal corneas, irregular corneal astigmatism,
519 asymmetry on corneal topography, against-the-rule astigmatism or steeper than normal
520 corneas. This approach has to be weighed by the risk of lower refractive predictability and
521 higher adverse effects hazard ratio (infectious keratitis, corneal haze). Despite a recent meta-
522 analysis on 1189 eyes [131], no consensus exists concerning this approach to date and it is
523 still unclear if the additional CXL step reduces the incidence of iatrogenic keratectasia.

524 *3. Photorefractive intrastromal CXL (PiXL)*

525 Recently, recent developments concerning CXL demonstrated greater reduction in
526 keratometry and corneal aberrations following cone-localized smaller diameter treatments in
527 comparison with the conventional broad-beam profile in keratoconic eyes. Some authors
528 extrapolated this principle for normal nonectatic corneas, in which smaller diameter high-
529 fluence irradiation is used to selectively target the central cornea to produce a central
530 flattening, with a subsequent myopic correction. Several studies reported promising results on
531 low myopia (spherical equivalent: manifest refraction spherical equivalent of -3.00 diopters
532 or less) in treating low myopia with epithelium-off [132] or epithelium-on [133] CXL as an
533 alternative to photoablation. Epithelium-on PiXL could represent a novel approach for
534 myopic correction as a mini-invasive, nonincisional, nonablative treatment modality for
535 myopic correction.

536 *4. Photoactivated chromophore CXL for infectious keratitis (PACK-CXL)*

537 In vitro studies have shown CXL to be effective against many pathogenic microorganisms
538 responsible for infectious keratitis [134,135] as well as to improve the corneal resistance to
539 degradation processes and enzymatic digestion and to prevent corneal melting [14]. Since,
540 several studies and case reports reported interesting results of CXL in the treatment of
541 recalcitrant or severe infectious keratitis [136–140]. To date, three prospective clinical trials
542 have been conducted on this topic. In 2014, a first prospective clinical trial [141] on 40 eyes
543 showed that CXL is an effective adjuvant therapy in severe bacterial or fungal keratitis with
544 corneal melting as it lowered the complication and perforation rate although it did not shorten
545 the time to corneal healing when compared to control. Encouraging results were found by
546 Bamdad et al [142] in another clinical trial on 32 eyes with moderate bacterial keratitis and
547 showed significant shorter healing time in CXL group. However, in 2015, a third randomized
548 clinical trial including 13 eyes with recalcitrant deep stromal fungal keratitis was prematurely

549 stopped because of a higher rate of perforation and worse visual outcomes when compared to
550 control. Interestingly, ulcers caused by *Aspergillus* species had a poorer healing response
551 versus *Fusarium* species. Unfortunately, these studies are under-powered and no definitive
552 conclusions can be drawn about the efficacy of PACK-CXL, especially on severe fungal
553 keratitis. Although most of authors considered CXL as an adjuvant therapeutic tool in
554 infectious keratitis, Makdoui et al [143] conducted a prospective non-randomized study
555 with promising results on 16 eyes treated with CXL as the primary therapy for bacterial
556 keratitis, but this latter approach remains anecdotic to date. In this study, only 2 eyes (12.5%)
557 required topical antibiotics adjunction. The effectiveness of PACK-CXL on acanthamoeba
558 keratitis is more controversial. Some clinical studies reported interesting outcomes of epi-off
559 CXL on acanthamoeba keratitis. [144,145] However, several in vitro studies have shown that
560 riboflavin mediated CXL did not confer any positive effect on acanthamoeba cyst or
561 trophozoites [146,147] whereas Rose Bengal mediated CXL did. [146]. A recent meta-
562 analysis concluded that adjuvant PACK-CXL expedites the healing when compared to
563 standard antibiotics care but given the limitations of the existing studies about PACK-CXL in
564 treating infectious keratitis in the literature, a larger scale, well-designed randomized clinical
565 trial is needed to fully assess the efficacy and safety of this approach, especially in fungal
566 keratitis. PACK-CXL should not be employed to treat viral keratitis as UV radiation may
567 exacerbate or activate herpes simplex infection. [148–150].

568 5. *CXL for bullous keratopathy*

569 Bullous keratopathy (BK) results from endothelial dysfunction leading to corneal
570 decompensation, corneal edema and opacification and epithelial bullae formation. Hence, BK
571 is responsible for chronic ocular pain as well as visual impairment. Corneal transplantation is
572 the gold standard treatment for BK, however, donor corneas are not always readily available
573 and waiting period can last several months. CXL induced chemical bonds between amino-

574 terminals of the collagen side chains and the proteoglycans of the extracellular matrix that
575 creates a mechanical barrier, decreased stromal imbibition pressure and reduced
576 transendothelial inflow, thereby increasing corneal transparency [151]. Ehlers et al. [151]
577 were the first to use CXL in corneal edema for endothelial decompensation. In a preliminary
578 study published in 2008, they used C-CXL protocol in 11 eyes with promising improvement
579 on visual acuity and ocular pain. In 2009, Wollensak et al [152] showed that C-CXL
580 significantly improved ocular pain and discomfort, visual acuity and corneal edema up to 8
581 months postoperatively. In a prospective study on 14 eyes with BK, Ghanem et al [153]
582 showed similar results on corneal transparency, corneal thickness, and ocular pain 1 month
583 postoperatively using C-CXL protocol, but the effect was transient and lasted for less than 6
584 months. In 2014, Sharma et al [154] confirmed these results on ocular pain in a retrospective
585 noncomparative case series, with 44% of bullae recurrences at 6 months. Several other studies
586 [155,156] confirmed that epi-off CXL reduces symptoms of bullous keratopathy, and could be
587 beneficial as a temporary measure for patients awaiting or denied for corneal transplant.

588

589 **Conclusion**

590 CXL treatment is currently the sole treatment that aims at strengthening the cornea and
591 stopping progression of keratectasia. Although a considerable number of studies are reported
592 in the literature concerning CXL, some discrepancies and unresolved issues remain. In any
593 cases management involves controlling risk factors such as eye rubbing and atopy. If long-
594 term studies showed that C-CXL was effective in halting keratoconus, exact efficacy of newer
595 protocols remains unclear. Considering this up-to-date review, a pragmatic clinical approach
596 can be proposed. C-CXL should be preferred in aggressive and rapidly progressive cases. A-
597 CXL could be considered in documented progression in most of cases. When time exposure is
598 minimal, A-CXL should be enhanced as in pulsed or oxygen enrichment procedures. In

599 advanced cases with thin corneas (CCT below 400 μm) or in an effort to minimize
600 iatrogenicity in specific cases, I-CXL could be considered as a less-invasive alternative,
601 keeping in mind the higher risk of failure. In case of pediatric keratoconus or poor compliance
602 in eye rubbing avoidance (i.e. autistic condition), a careful and close monitoring is mandatory.
603 Various treatment protocols are currently in development, bringing the hope of less invasive
604 approaches and promising refinements and improvements in keratoconus management.

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1100 Pediatric Keratoconus: Long-Term Results. *Cornea*. 2016;35:954–8.
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1104 **Figures legends**

1105 **Figure 1:** *In-vivo* confocal microscopy aspect of the sub-basal nerve plexus and mid-stromal
1106 keratocytes of the cornea following epithelium-off accelerated-CXL procedure (10 minutes, 9
1107 mW/cm²) showing:

1108 **A:** Pre-operative aspect of the sub-basal nerve plexus showing distorted nerves along with
1109 numerous anastomoses with characteristic Y shaped bifurcations and H shaped
1110 interconnections (white arrows); **B:** 1 month post-operatively, showing early and complete
1111 obliteration of the sub-basal nerve plexus (white arrows); **C:** 6 months post-operatively,
1112 showing neuroregeneration of the sub-basal nerve plexus which appears fragmented. Nerves
1113 are less tortuous when compared to baseline (white arrows); **D:** 12 months post-operatively,
1114 showing normal keratocytes density and complete neuroregeneration. A complete
1115 interconnected network is visible but less anastomosis, bifurcations, interconnections and
1116 tortuosity are noted when compared to baseline (white arrows); **E:** Pre-operative mid-stroma
1117 aspect showing numerous keratocytes as hyper-reflective white- rounded structures (white
1118 arrows); **F:** 1 month post-operatively, showing decrease in keratocytes density along with
1119 stromal fibrosis (white arrows) and honeycomb-like structures (black arrow); **G:** 6 months
1120 post-operatively, few keratocytes are visible and less fibrosis is visible but keratocyte density
1121 remains inferior to baseline (white arrows); **H:** 12 months post-operatively, showing
1122 keratocytes repopulation (white arrows) with diminution of stromal fibrosis and a similar
1123 keratocyte density when compared to baseline.

1124

1125 **Figure 2:** AS-OCT at 1 month postoperatively showing the demarcation line as a hyper-
1126 reflective line within the stromal bed at various depths. **A:** Standard Dresden epithelium-off
1127 procedure (C-CXL); **B:** Epithelium-on transepithelial CXL procedure with iontophoresis (I-
1128 CXL); **C:** Epithelium-off accelerated-CXL procedure at 9 m W/cm² for 10 minutes (A-CXL).

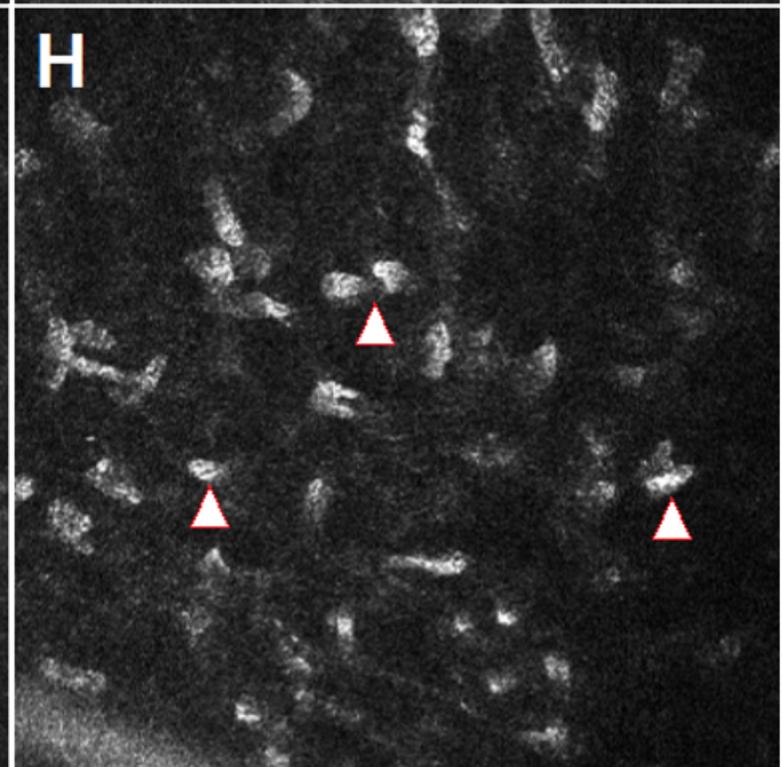
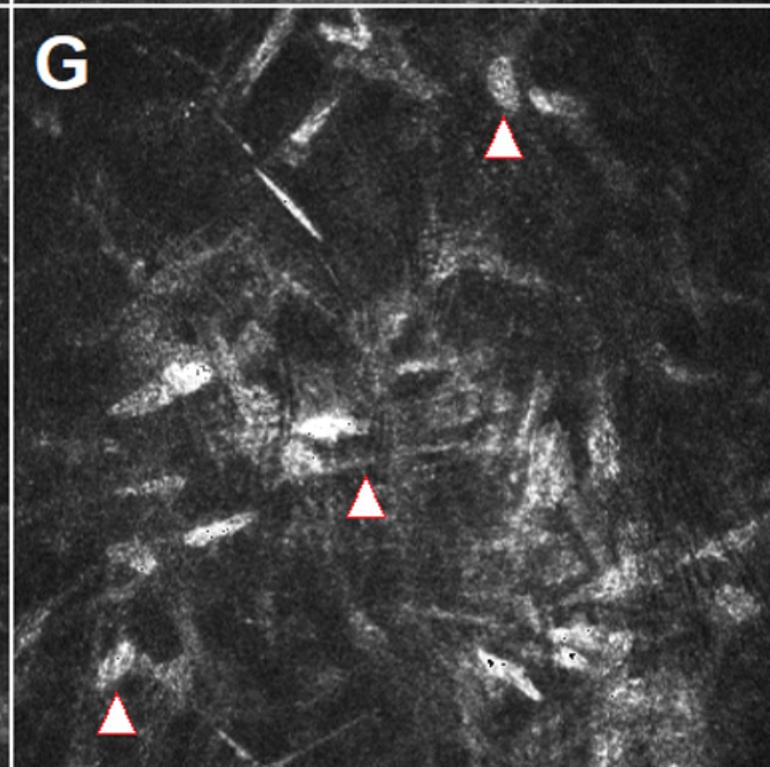
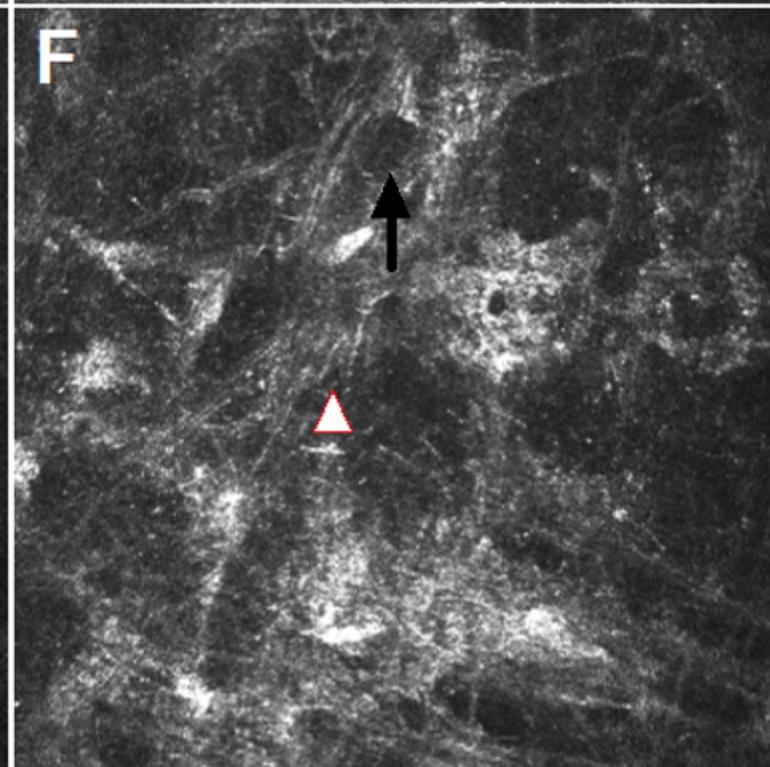
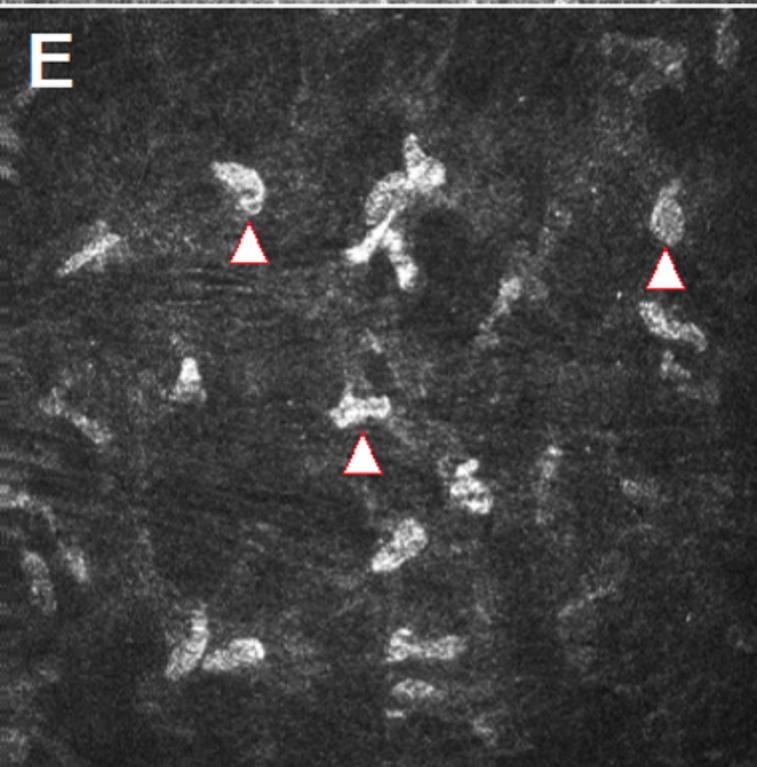
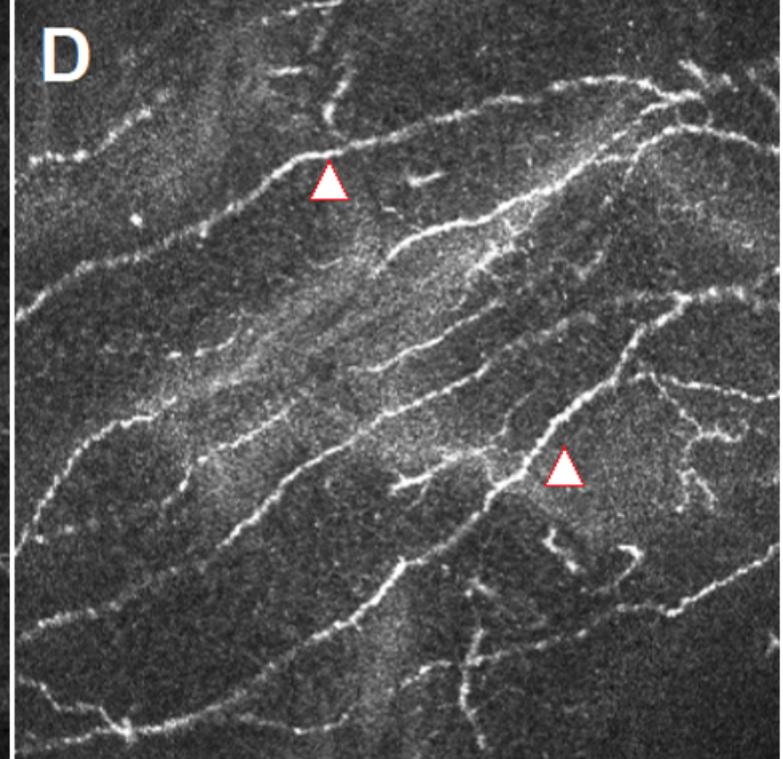
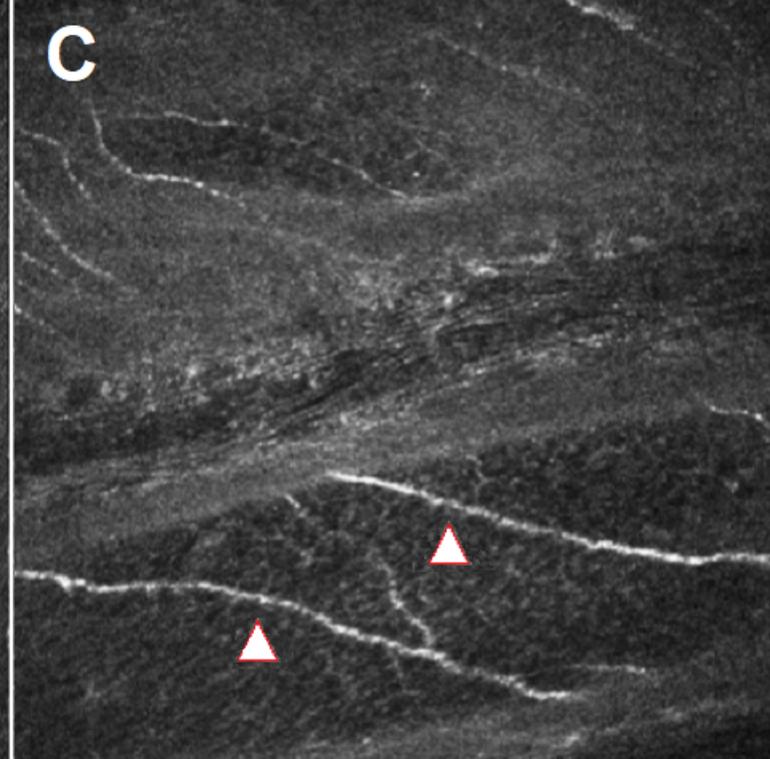
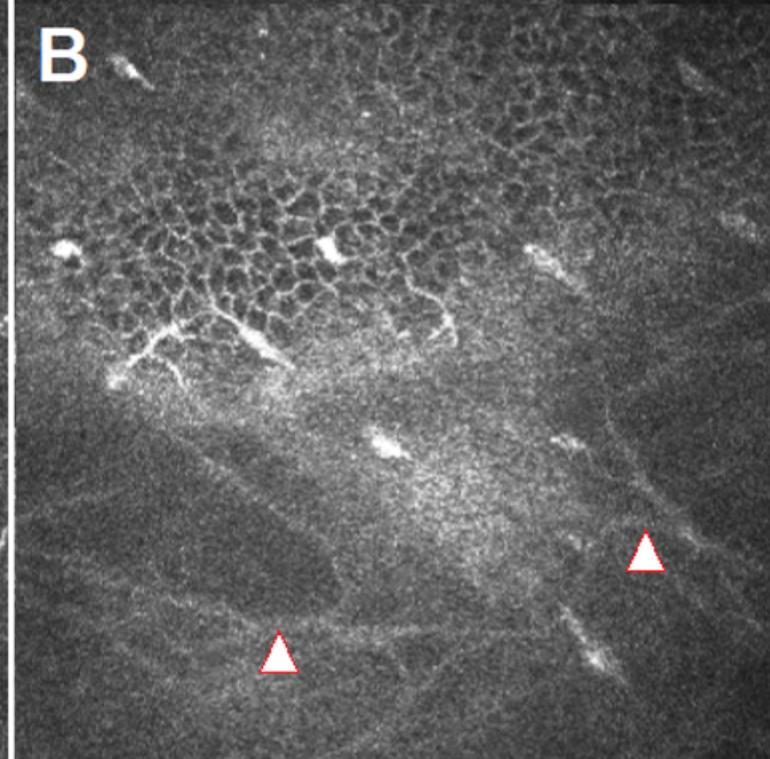
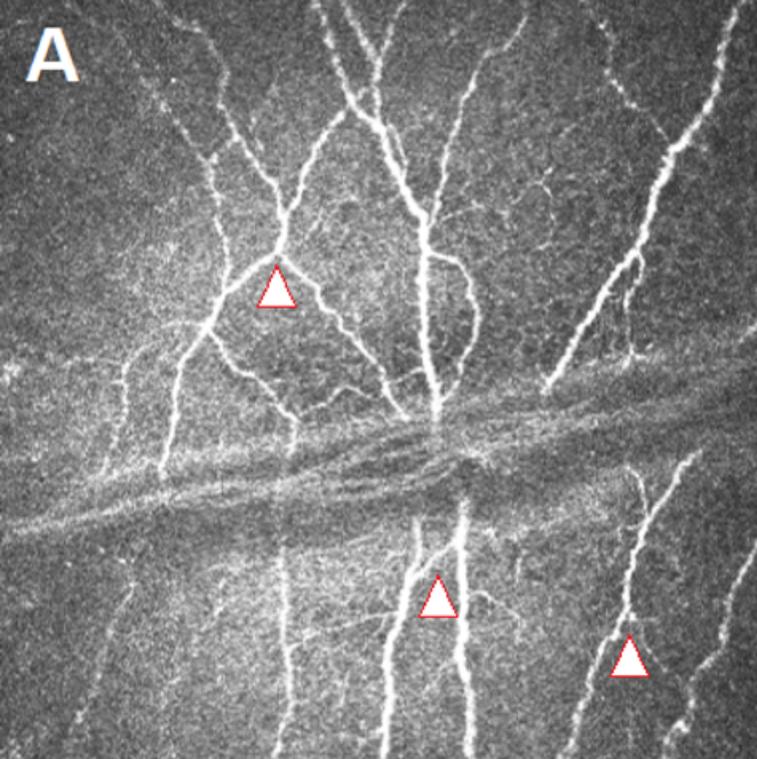
1129 **Figure 3:** Change in epithelial thickness mapping from baseline to 12 months postoperatively
1130 showing typical inferotemporal postoperative epithelial thinning after epithelium-off A-CXL
1131 protocol (**A:** baseline; **B:** 12 months postoperatively)

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1133 **Figure 4:** Topographic aspect following combined CXL-plus double procedure: CXL and
1134 topoguided-phototherapeutic keratectomy. **A:** Pre-operative sagittal keratometric curvature
1135 mapping; **B:** Post-operative sagittal keratometric curvature mapping; **C:** Differential sagittal
1136 keratometric curvature mapping; **D:** Excimer laser topoguided-ablation profile showing
1137 maximal keratometric improvement of 5.3 diopters and corneal regularization.

1138

1139 **Figure 5:** Topographic aspect following combined CXL-plus triple procedure: CXL,
1140 intracorneal ring segment implantation (ICRS - 320°, 250 µm depth) and topoguided-
1141 phototherapeutic keratectomy (TG-PTK). **A:** Pre-operative sagittal keratometric curvature
1142 mapping; **B:** Post-operative sagittal keratometric curvature mapping following ICRS
1143 implantation; **C:** Post-operative sagittal keratometric curvature mapping following TG-PTK;
1144 **D:** Differential sagittal keratometric curvature mapping following ICRS implantation versus
1145 baseline; **E:** Differential sagittal keratometric curvature mapping following TG-PTK versus
1146 **D;** **F:** Differential sagittal keratometric curvature mapping following the triple procedure
1147 versus baseline; **G:** Excimer laser topoguided-ablation profile showing maximal keratometric
1148 improvement of 14.7 diopters and corneal regularization.



Pre-operative (A-CXL)

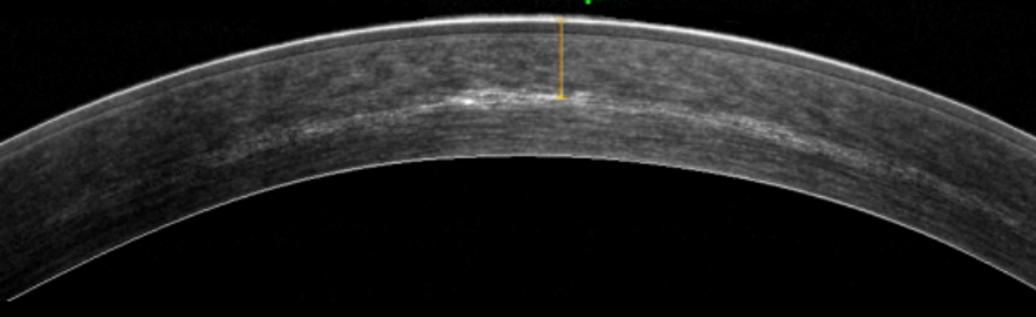
1 month

6 months

12 months

A: C-CXL

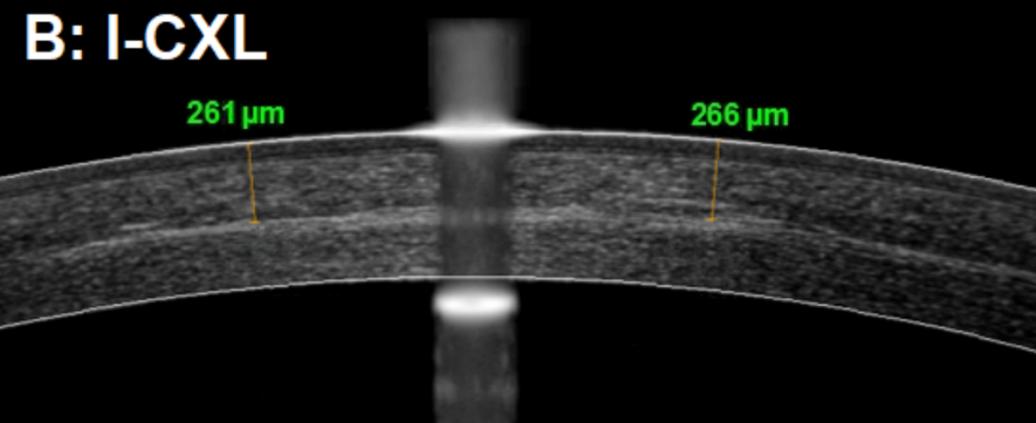
307 μm



B: I-CXL

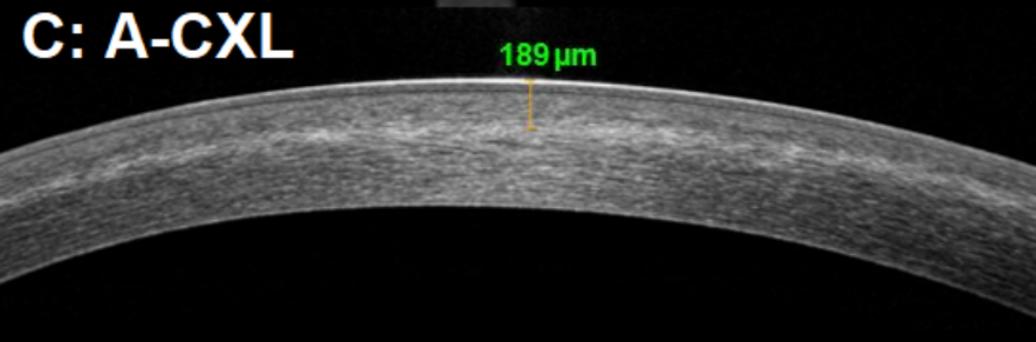
261 μm

266 μm

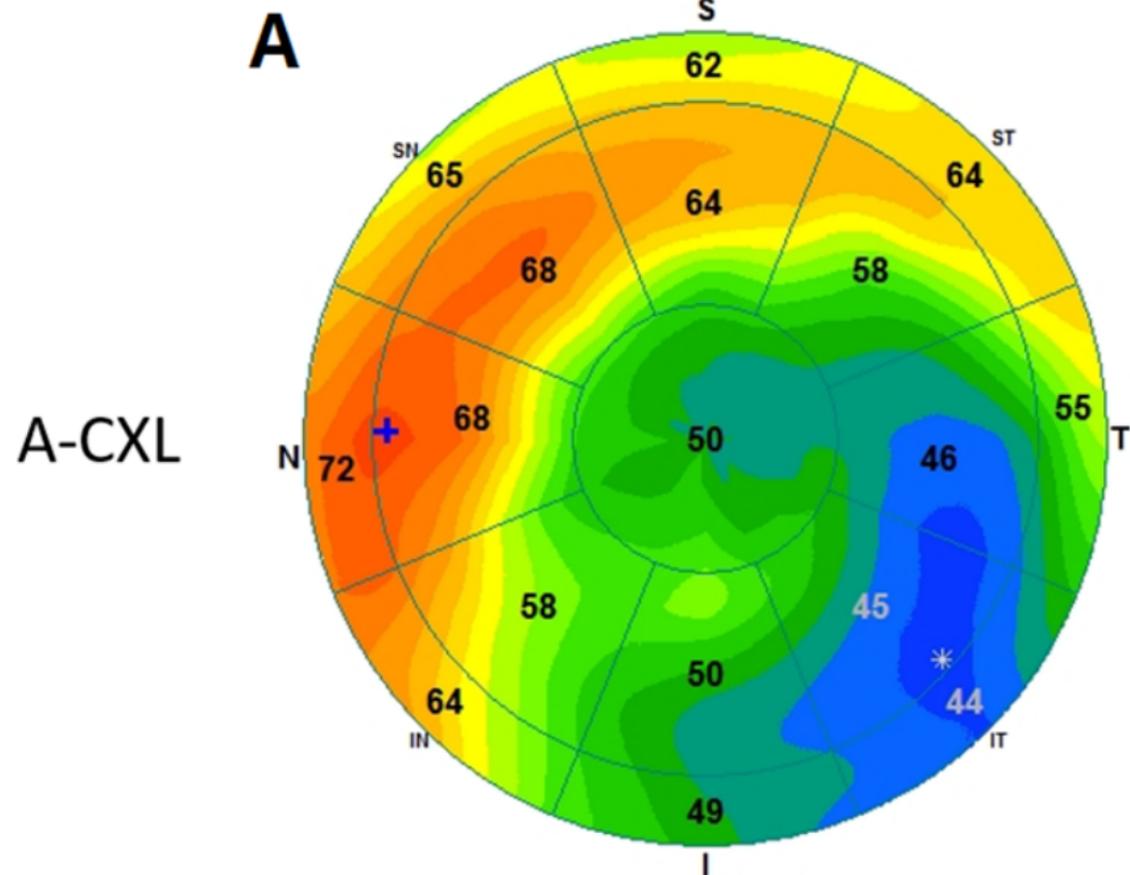


C: A-CXL

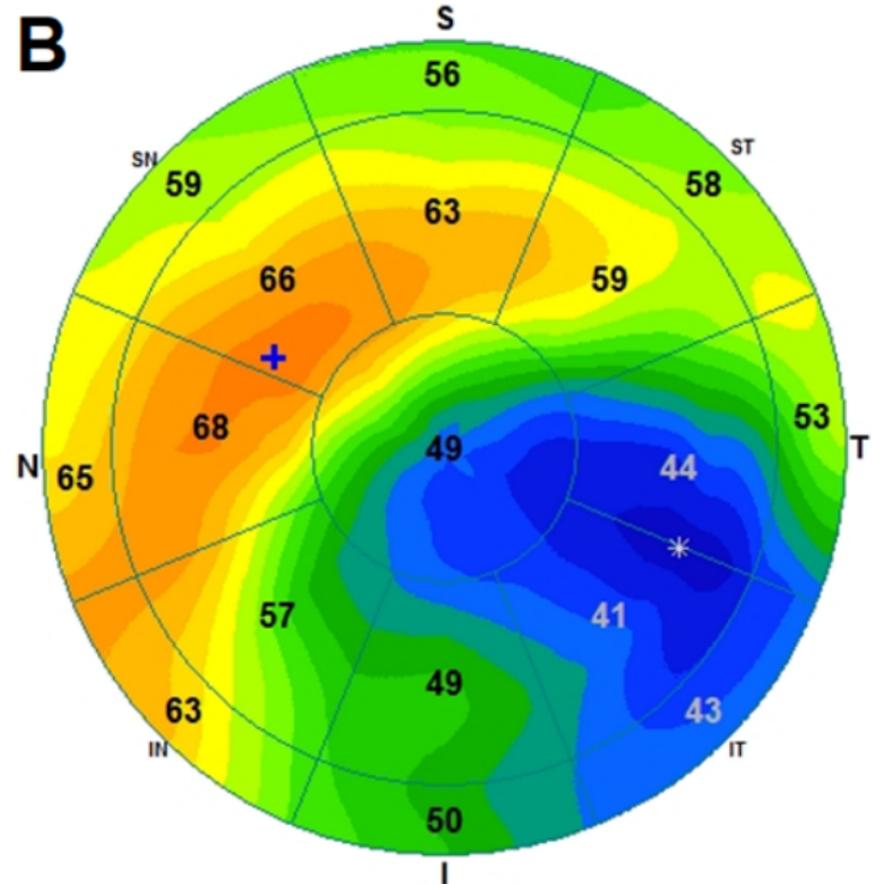
189 μm



Pre-CXL (epithelial map)



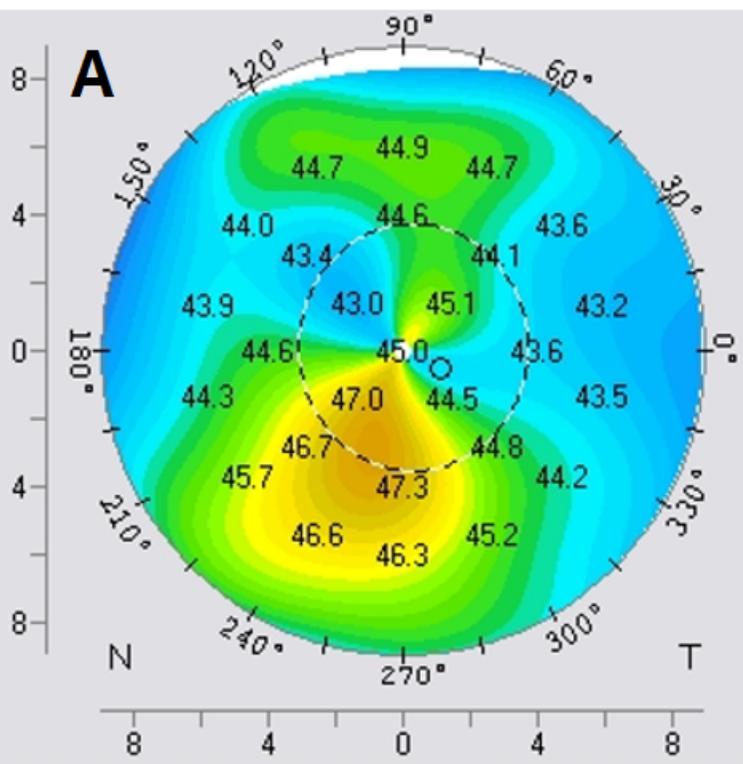
Post-CXL (12 months)



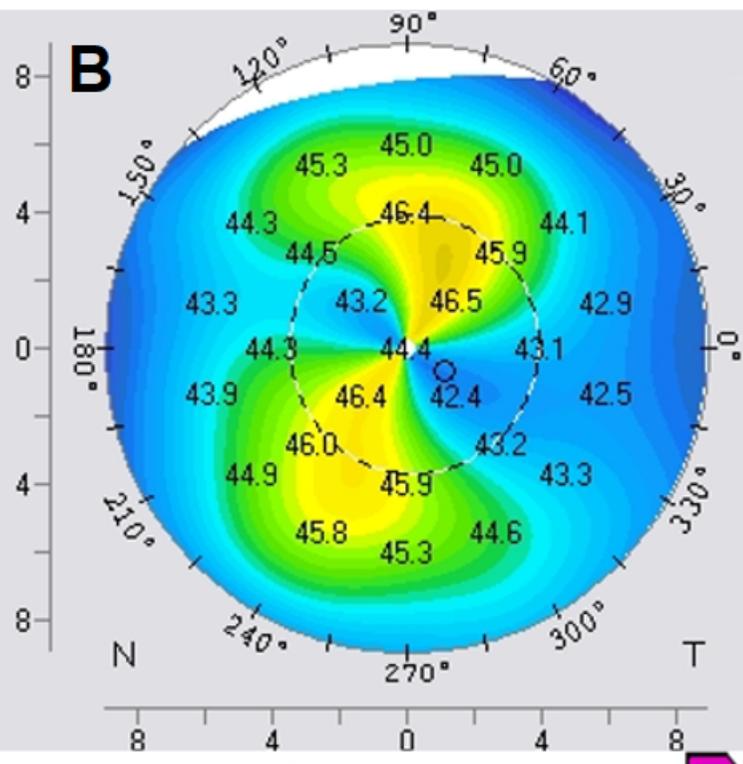
Pre-operative

Post-excimer laser

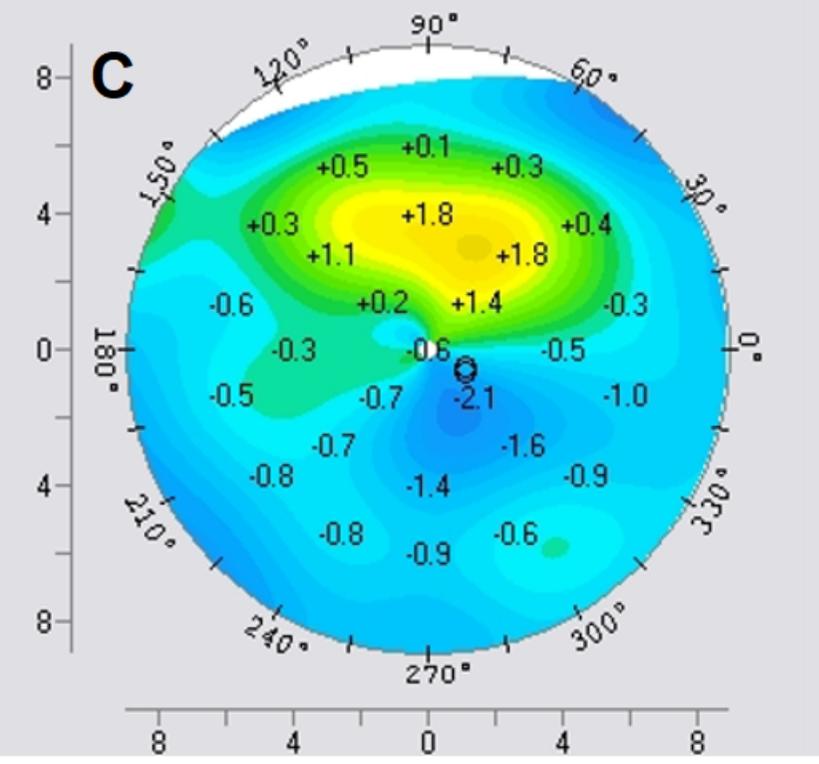
A



B

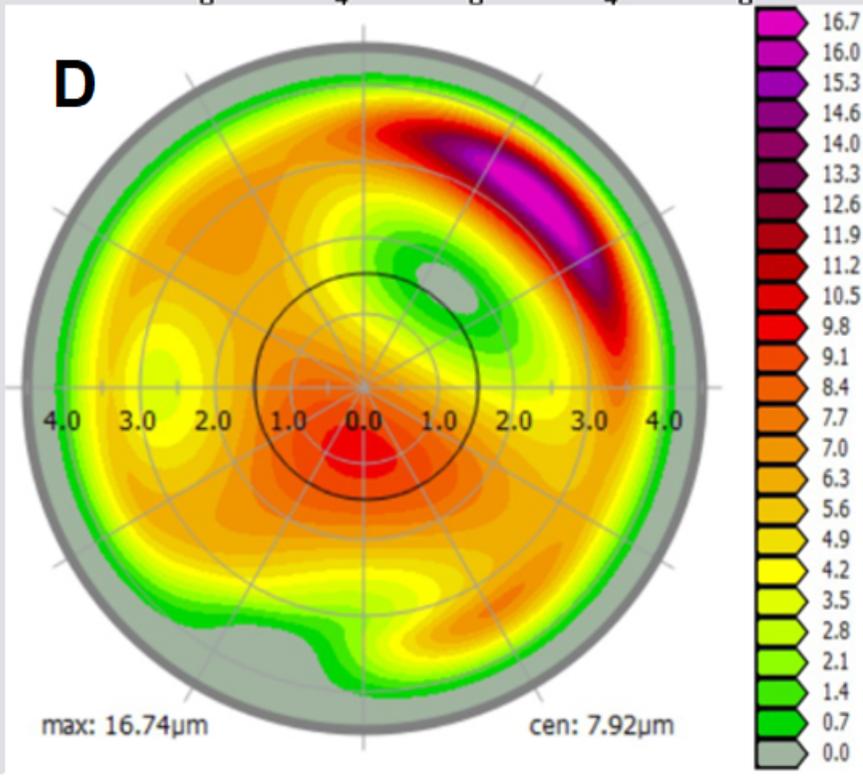


C



Excimer laser effect

D



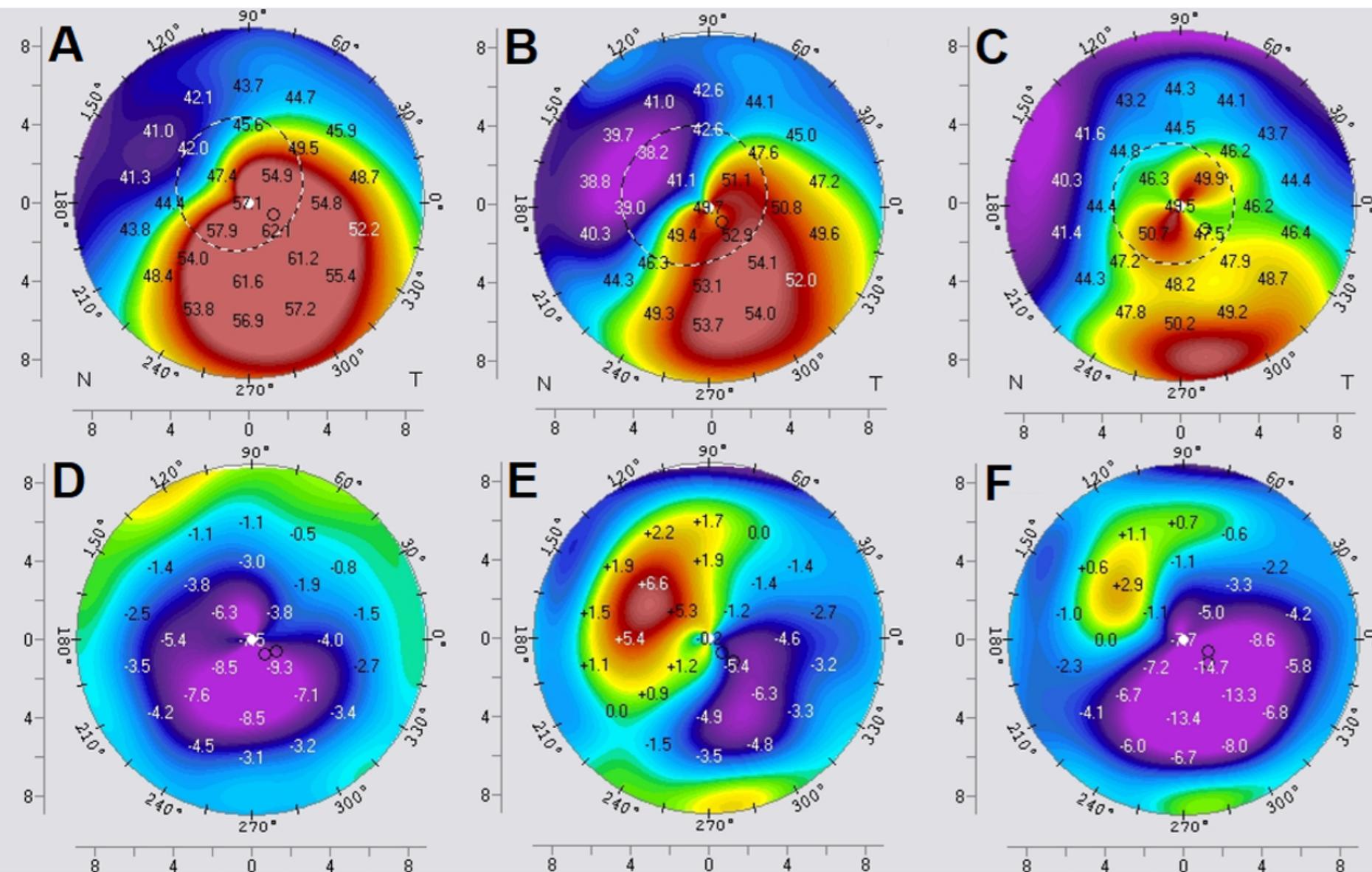
Excimer laser ablation profile

Pre-operative

Post-corneal ring

Post-excimer laser

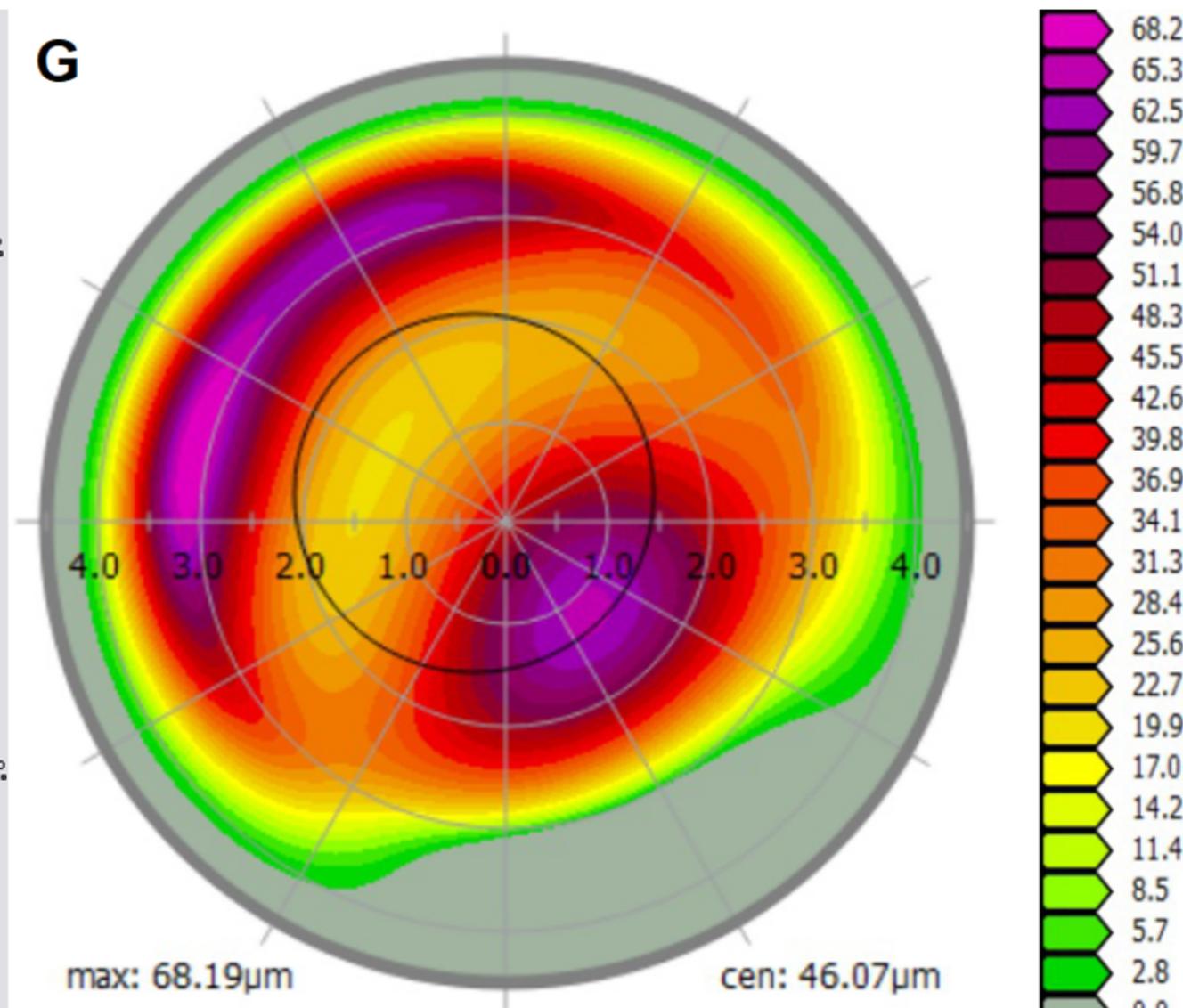
Excimer laser ablation profile



Corneal ring effect

Excimer laser effect

Total effect

max: 68.19 μm cen: 46.07 μm

First author (year)	Design	CXL protocol	Nb eyes	Follow-up (months)	Failure rate (>1D)	Conclusion
Dervenis (2020) [157]	RNR	pA vs C	19 vs 40	7	NA	pA = C
Ziaei (2020) [158]	PNR	C, A, TE pA	40 vs 40 vs 40	24	0%, 5%, 3.5%	Effective
Farhat (2020) [159]	RNR	C	156	36	19.87%	Effective
Hatch (2020) [160]	PNR	A	612	11	17.9%	Effective
Nicula (2019) [161]	RNR	C	113	120	0 %	Effective, stable
Singal (2019) [129]	PNR	A	204	12	21%	Effective
Lombardo (2019) [162]	PR	I vs C	22 vs 12	24	10% vs 0%	C > I
Liao (2019) [163]	PNR	I 5min vs 10min	42	12	27.8% vs 16.7%	10 min > 5 min
Hersh (2018) [164]	PNR	TE (1 min vs 2 min)	44 vs 38	12	6.8% vs 23.6%	Effective
Kuechler (2018) [165]	RNR	C	61	24	18%	Effective
Stulting (2018) [71]	PNR	TE	512	24	8.3%	Effective
Huang (2018) [166]	PNR	TE	25	24	28%	Effective
Toker (2017) [167]	RNR	C vs A (9mW) vs A (30mW) vs pA (30mW)	34 vs 45 vs 28 vs 27	12	6% vs 7% vs 11% vs 11%	C = 9mW but > 30mW
Hersh (2017) [168]	PR	C vs sham	91 vs 88	12	14.5%	Effective
Jouve (2017) [64]	PNR	C vs I	40/40	24	7.5% vs 20%	C > I
Lombardo (2017) [62]	PR	I vs C	22 vs 12	12	5% vs 8%	C > I
Cantemir (2017) [169]	RNR	I vs C	40 vs 40	36	2.5% vs 0%	I = C
Bikbova (2016) [170]	PR	I vs C	73/76	24	1.3% vs 0%	C > I
Syedean (2015) [171]	PR	C vs contralateral control	26 vs 26	12	12% vs 38.5%	Effective
Poli (2015) [172]	PNR	A	36	72	11%	Effective
Soeters (2015) [58]	PR	TE vs C	35 vs 26	12	23%	C > TE
Shalchi (2015) [112]	Review	Epi-off vs epi-on	>20	>12	8.1–33.3%	Effective

Table 1: CXL protocols efficacy in keratoconus (adults)

PR: prospective randomized; PNR: perspective non-randomized; RNR: retrospective non-randomized; NA: non-available; pA: pulsed-accelerated; CXL; A: accelerated CXL; C: standard CXL; TE: transepithelial CXL; I: iontophoresis transepithelial CXL

$K_{max} \geq 1D$ increase
$K_{max} - K_{min} \geq 1D$ increase
$K_{mean} \geq 0.75D$ increase
Pachymetry $\geq 2\%$ decrease in CCT
Corneal apex power $> 1D$ increase
MRSE $> 0.5D$ increase

Table 2: Criteria for keratoconus progression vs baseline measurement

CCT: central corneal thickness; D: diopters; Kmax: steepest keratometry; Kmin: flattest keratometry; MRSE: manifest refractive spherical equivalent; $K_{mean} = (K_{max} + K_{min})/2$; Corneal apex power is measured with cone location and magnitude index

First author (year)	Design	CXL protocol	Nb eyes	Follow-up (months)	Failure rate (>1D)	Conclusion
Tian (2020) [173]	PNR	A-TE	53	36	20%	Effective
Iqbal (2020) [174]	PR	C vs A vs TE	91/92/88	24	0% vs 5.4% vs 28.4%	Effective
Agca (2020) [175]	RNR	30 mW (A) vs 18 mW (A)	30 vs 113	60	23% vs 17%	Efficacy ↓ if fluence ↑
Turhan (2020) [176]	RNR	C vs A	26 vs 22	24	7.8% vs 18.2%	Effective
Nicula (2019) [177]	RNR	A vs C	27 vs 37	48	7.4% vs 0%	Effective
Buzzonetti (2019) [178]	RNR	I vs C	20/20	36	25% vs 50%	C > I
Mazzotta (2018) [179]	PNR	Siena	62	120	24%	Effective, stable
Sarac (2018) [180]	RNR	C vs A	38 vs 49	24	13.1% vs 16.3%	Effective
Henriquez (2018) [181]	PNR	C	26	36	23%	Effective
Henriquez (2017) [182]	PNR	A-TE vs C	36 / 25	12	5.6% vs 12%	A-TE = C
Godefrooij (2016) [183]	PNR	C	54	60	22%	Effective, stable

Table 3: CXL protocols efficacy in keratoconus (pediatric)

PR: prospective randomized; PNR: perspective non-randomized; RNR: retrospective non-randomized; NA: non-available; A: accelerated CXL; C: standard CXL; TE: transepithelial CXL; I: iontophoresis transepithelial CXL