CLINICAL INVESTIGATION

Cyclosporine ophthalmic emulsions for the treatment of dry eye: a review of the clinical evidence

Dry eye has gained recognition as a public health problem given its high prevalence, morbidity and cost implications. Although dry eye is common and affects patients' quality of life, only one medication, cyclosporine 0.05% emulsion, has been approved by the US FDA for its treatment. In this review, we summarize the basic science and clinical data regarding the use of cyclosporine in the treatment of dry eye. Randomized controlled trials showed that cyclosporine emulsion outperformed vehicles in the majority of trials, consistently decreasing corneal staining and increasing Schirmer scores. Symptom improvement was more variable, however, with ocular dryness shown to be the most consistently improved symptom over vehicle.

Philip Ames[1,2] & Anat Galor*[,1,2]

[1]Miami Veterans Administration Medical Center, 1201 NW 16th St, Miami, FL 33125, USA

[2]Bascom Palmer Eye Institute, University of Miami, 900 NW 17th Street, Miami, FL 33136, USA

*Author for correspondence:
Tel.: +1 305 326 6000
Fax: +1 305 575 3312
agalor@med.miami.edu

Dry eye is a multifactorial disorder of the ocular surface involving the tear film and the reflex control of tear homeostasis. There are two major forms of dry eye: lacrimal-deficient or aqueous-deficient dry eye (ADDE) and evaporative dry eye (EDE). In the United States and worldwide, dry eye has been estimated to affect 5–30% of the population [1]. Patients with dry eye complain of a variety of symptoms including poor visual quality, pain (burning, aching) and tearing. Symptoms associated with dry eye are a leading cause of visits to eye clinics and its treatment has significant cost implications [2,3]. Dry eye adversely impacts quality of life as its symptoms interfere with activities of daily living such as driving, reading and watching television. Studies using the Impact of Dry Eye on Everyday Life questionnaire have confirmed that dry eye negatively affects physical and mental functioning [4,5].

Symptoms & signs of dry eye

Despite its high frequency and morbidity, there is no gold standard for dry eye diagnoses. As such, most clinicians rely on a combination of symptoms and signs to detect and monitor the disorder. Several questionnaires are available to document dry eye symptom severity, the most popular being the ocular surface disease index (OSDI). This questionnaire consisting of 12 questions with possible scores ranging from 0 (no symptoms) to 100 (maximal symptoms) [6]. Common tear film and ocular surface assessments in dry eye include tear breakup time (TBUT) [an assessment of tear film stability, lower scores are indicative of tear instability], corneal staining [an assessment of corneal epithelial cell disruption, higher scores indicative of more disruption], basal or reflex tear secretion test (Schirmer's strips) [an assessment of tear secretion, lower scores are indicative of less secretion], and morphologic and qualitative characterization of the eyelid margin and meibomian glands. Newer tests that can provide subclinical information on the ocular surface environment have more recently become available including measurement of tear osmolarity (TearLAB, CA, USA) and of tear MMP-9 as an index of ocular surface inflammation (Inflammadry, RPS, Tampa, FL, USA).

Unfortunately, many groups have demonstrated poor correlation between dry eye symptoms and signs [7,8], a fact that makes diagnosing, treating and researching dry eye challenging. Even when separately measuring the two major subtypes of dry eye – ADDE and EDE, neither were significantly correlated with the presence of symptoms [7,8]. It

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can also be difficult to appraise certain tests like osmolarity. There is currently no commercially available way to measure osmolarity in the central cornea which is believed to greatly exceed the osmolarity levels found in the inferior tear meniscus and be responsible for the discomfort symptoms [7,8]. Likewise, there are likely unmeasured factors in dry eye, such as ocular sensory apparatus function, that may become sensitized in patients with ocular surface inflammation and high osmolarity.

Inflammation & dry eye

It is well recognized that inflammation plays an important role in dry eye. Early studies demonstrated that patients with dry eye had increased $CD4^+$ T cells and HLA-DR expression in their conjunctivae and higher levels of inflammatory mediator expression like ICAM-1 [9–11]. A classic paper that established this concept was published by Niederkorn *et al.* in 2006 [12]. In this paper, mice were first subjected to a low humidity environment and were given scopolamine which causes decreased aqueous tear production. These experimental conditions led to the development of T-cell-mediated inflammation on the ocular surface with clinical manifestations that resembled dry eye in humans (i.e. corneal staining). The authors were then able to induce a similar disease picture in nude mice by adoptively transferring $CD4^{(+)}$ T cells from the affected animals [12].

Since then, many experimental models have expanded on this concept and have found that other parts of the immune system, like antigen presenting cells and immunoglobulins, are also important in the development of experimental dry eye [13,14]. In mice, several therapies that interfere with the inflammatory cascade, like IL-17 [15], IL-1 [16] and chemokine receptor 2 [17] inhibition, were found to improve experimental dry eye.

Inflammation is also a component of dry eye in humans [18,19]. As above, T cells have been described in the conjunctivae [11] of patients with dry eye and elevated levels of various inflammatory cytokines have been found in their tears [18,20]. Specifically, tear levels of IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-17, TNF-α and IL-6 have all been found to be elevated in dry eye compared with control subjects [18,20]. It is not clear, however, what percentage of patients with dry eye symptoms have underlying ocular surface inflammation and to what degree this inflammation drives symptoms.

Cyclosporin's mechanism of action, basic pharmacology & pharmacokinetics

Given the role of inflammation in dry eye, it makes sense that anti-inflammatory agents have been evaluated in its treatment. Cyclosporine (CsA) emulsion 0.05% (Restasis®, Allergan, CA, USA) has been the only product to receive US FDA approval for the treatment of dry eye.

Mechanism of action

CsA is an immunosuppressant medication that was originally used to prevent rejection after organ transplantation. It affects immune function by interfering with the activity and growth of T cells (Figure 1). In the normal situation, T-cell receptor activation leads to the influx of calcium (Ca^+) into the cytoplasm. Intracellular calcium binds the cytosolic protein calmodulin, which in turn binds and activates calcineurin. This calmodulin/calcineurin complex then dephosphorylates the transcription factor nuclear factor of activated T cells (NFATc), which translocates into the nucleus and increases the activity of genes coding for IL-2 and other inflammatory cytokines. CsA exerts its action after it enters the cytoplasm of T cells and binds to cyclophilin. The CsA/cyclophilin complex affects T-cell activity by blocking the action of calcinuerin and preventing NFATc dephosphorylation. The subsequent reduction in IL-2 levels also reduces the function of effector T cells.

CsA can also affect mitochondrial activity in some cells. In human conjunctival epithelial cells, the inflammatory mediators TNF-α and IFN-γ induce mitochondrial permeability transition pore (MPTP) opening, upregulate Fas/FasL and caspase, and increase cell apoptosis. CsA prevents epithelial cell death by blocking MPTP opening, Fas/FasL and caspase activation [21]. Interestingly, a similar effect of CsA on blocking MPTP opening was not seen in activated T cells [21].

Formulation

CsA has poor solubility in water, and, as a consequence, suspension and emulsion forms are needed. The current pharmaceutically available product, Restasis, is formulated with 0.05% CsA in a homogenous emulsion of glycerin (2.2%), castor oil (1.25%), polysorbate 80 (1.00%), carbomer copolymer type A (0.05%), purified water (to 100%) and sodium hydroxide for pH adjustment [22]. However, CsA can be compounded into other strengths, the most common being 0.5, 1 and 2%. The lower doses (0.5, 1%) are often compounded using injectable CsA in artificial tears. The higher dose (2%) is often compounded using the oral solution of CsA in sterile olive or corn oil. This is because the alcohol content in the injectable is very high and it makes it very hard to tolerate in the 2% formulation [23]. These latter formulations are more likely to be used in countries where Restasis is not available.

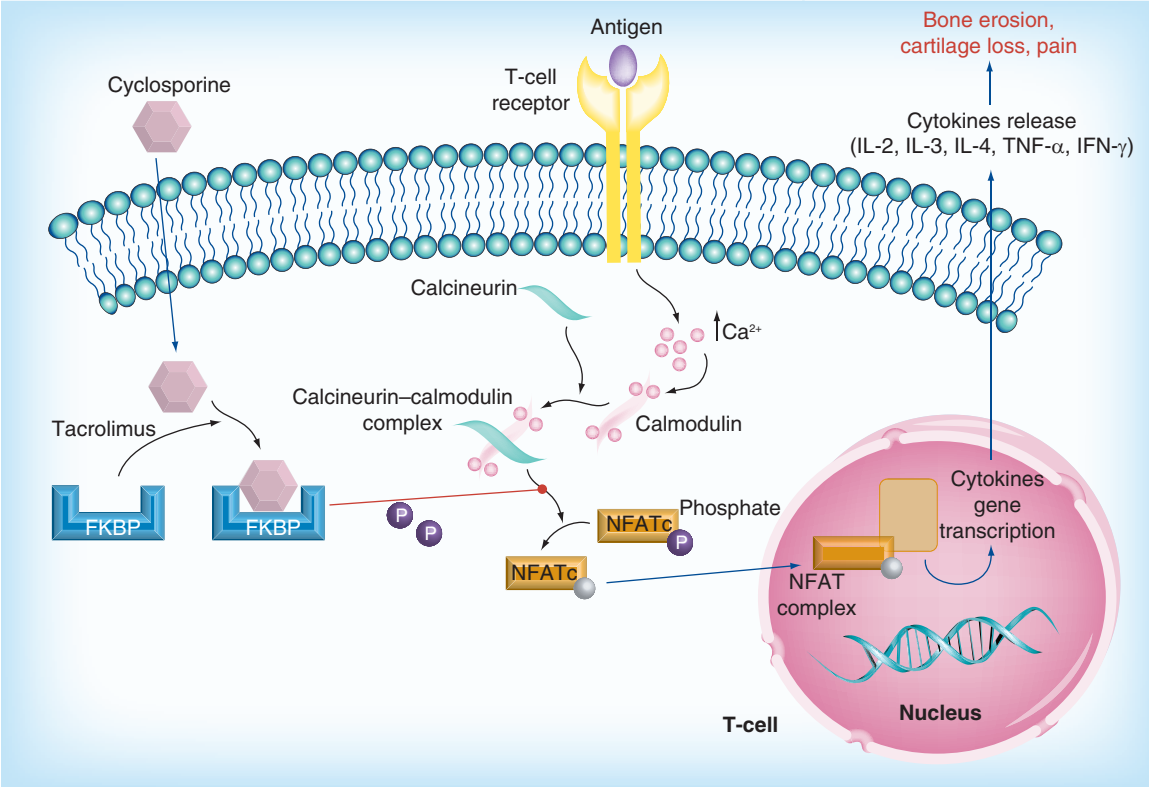

Figure 1. Cyclosporine acts by forming a complex with intracellular binding proteins after entering T cells. This complex inhibits calcinuerin phosphatase, halting the activation of the transcription factor NFATc, thus preventing the production of cytokines including IL-2 and IFN-γ.

Blood concentration

No quantifiable CsA levels were found in the blood of patients treated with 0.05% CsA [24,25]. A few patients treated with 0.1% CsA (n = 6) had detectable CsA blood levels although none exceeded 0.3 ng/ml [25].

Methods

A PubMed search was conducted using the terms 'CsA dry eye'. All searches were limited to the English language or English translation. Articles were reviewed and those that discussed basic science and human studies of CsA in the treatment of dry eye were summarized. For the human studies, all studies that evaluated the use of CsA in dry eye were included in this review. Human studies were assessed and ranked based on their level evidence in terms of masking and randomization.

Animal studies of CsA

Several animal models have looked at the effect of CsA in dry eye. Mice with a genetic mutation that rendered T cells autoreactive were treated systemically with 2-mg CsA daily from age 1–5 months. The degree of ocular and lacrimal gland disease was measured using scored histologic sections graded based on the presence or absence of mononuclear inflammatory cell foci. Additionally, planimetry was used to determine the percentage of area in the lacrimal gland that was involved with inflammatory cells. The results showed that CsA therapy markedly improved ocular and lacrimal gland inflammation with roughly a fivefold decrease in any ocular and lacrimal gland inflammation when compared with controls [26]. Another study which evaluated apoptosis in mice with desiccation-induced dry eye found less apoptotic cells in the conjunctival epithelium of animals treated topically with 0.05% CsA compared with vehicles or controls [27].

In seven normal rabbit eyes receiving a single instillation of 0.1% CsA, there was a significant increase in tear production (lacrimal gland fluid flow rate and Schirmer) and blink rate compared with vehicle. Although this could have been due to an irritant effect as sympathetically denervated eyes of rabbits from the same study showed significantly decreased blink rate and no effect on lacrimal gland tear fluid secretion when treated with CsA [28]. Another study in rabbits with induced autoimmune dacryoadenitis found significant decreases in $CD4^+$ lymphocytes in the lacrimal gland of 0.05% CsA topically treated animals versus vehicle [29]. In this group, Schirmer scores also slightly improved without significant changes in TBUT and staining.

Table 1. Summary of studies using cyclosporine for the treatment of moderate dry eye.

Study	Evidence level	N	Population	Dose/treatment length	Efficacy		Side effects	Ref.
					CsA versus control	CsA versus baseline†		
Chen *et al.*	Level 1A multicenter, randomized, double-blind, vehicle-controlled, parallel-group study	233	Patients with dry eye symptoms (≥2) and 2 of 3 (SchI ≤5, TBUT ≤5, PEE ≥1)	CsA 0.05% emulsion vs emulsion placebo vehicle plus AT (hypromellose) prn in both groups/8 weeks	CsA Better: Signs: corneal staining, SchI (4 wk); Symptoms: ocular dryness, total dry eye score, foreign body sensation. No difference: TBUT, photophobia, burning	Signs: conj hyperemia, TBUT; Symptoms: photophobia, burning	No differences between groups	[30]
Sall *et al.*	Level 1A multicenter, randomized, double-masked, parallel-group, 6-month, vehicle-controlled study	877	Patients with subjective dry eye (>3) and (SchI ≤5, PEE ≥5)	CsA emulsion (0.05 or 0.1%) vs emulsion placebo vehicle plus AT prn in all/6 months	CsA Better: Signs: corneal staining, Sch, global response to treatment; Symptoms: blurry vision (0.05%). No difference: dryness, sandy/gritty, itching, photophobia, burning and stinging, pain, ODSI and subjective facial expression	Signs: conj staining Symptoms: Blurry vision (0.1%), dryness, sandy/gritty, itching, photophobia, burning and stinging, pain, ODSI and subjective facial expression	Burning: 15% CsA (0.05%), 16% CsA (0.1%), 7% vehicle	[25]
Stevenson *et al.*	Level 1A randomized, multicenter, double-masked, parallel-group, dose-response controlled trial	162	Patients with dry eye symptoms (≥2) and (Sch1 <8, PEE ≥1)	CsA emulsion (0.05, 0.1, 0.2 or 0.4%) vs emulsion placebo vehicle plus Refresh (Allergan, CA, USA) prn in all/12 week	CsA Better: Signs: temporal conj staining (0.1% better than vehicle, 0.05%, 0.4%), SchI (0.4% better than vehicle, 0.2%); Symptoms: sandy/gritty (0.05 and 0.4% better than 0.2% and vehicle), ocular dryness (all CsA except 0.1% better than vehicle) No difference: itching (all CsA, better than baseline, vehicle was not), OSDI (0.1 and 0.2% better than baseline, 0.1% better than 0.2 and 0.05%)	Signs: temporal and nasal conj staining (all), TBUT (0.1% CsA) Symptoms: itching (all), ODSI (0.1%, 0.2% CsA; 0.1% better than 0.05%, 0.2%)	No serious events. Less ocular microbes recovered in CsA group	[35]

The studies have been cited in order of level of evidence. Each study has been categorized from Level 1 through 4 as follows: Level 1A – a randomized, double-masked design; Level 1B – a randomized, double-masked design, with weak patient masking due to significant variations in treatment versus placebo side-effect profiles; Level 2A – randomized‡, single-masked design; Level 2B – a randomized, single-masked design, with weak masking due to significant variations in treatment versus placebo side-effect profiles; Level 3 – randomized, non-masked; Level 4 – non-randomized, non-masked.
CsA (aqueous or emulsion) dosed twice a day unless otherwise noted.
†Signs and symptoms listed where CsA group had improved parameters compared with baseline but not compare to control.
‡Includes studies that could not be randomized because CsA was used in one eye and control was used in the other.
AT: Artificial tears; ATD: Aqueous tear deficiency; BAK: Benzalkonium chloride; CMC: Carboxymethylcellulose; conj: Conjunctivae; CsA: Cyclosporine; FU: Follow-up; IC: Impression cytology; LH: Lid hygiene; MGD: Meibomian gland dysfunction; OSDI: Ocular surface disease index; PEE: Punctate epithelial erosions; PF: Preservative free; Prn: As needed; Sch: Schirmer (mm); SchI: Schirmer's without anesthesia (mm); SchII: Schirmer's with anesthesia (mm); SS: Sjogren's syndrome; TBUT: Tear break up time(s); Vs: Versus; Wk: Week.

Table 1. Summary of studies using cyclosporine for the treatment of moderate dry eye (cont.).

Study	Evidence level	N	Population	Dose/treatment length	Efficacy		Side effects	Ref.
					CsA versus control	CsA versus baseline†		
Prabhasawat *et al.*	Level 1B prospective, randomized, double-masked, parallel-group controlled trial	70	Patients with symptoms and MGD (abnormal lid parameters, TBUT <8)	CsA 0.05% emulsion vs CMC (Cellufresh, Allergan, CA, USA) 2× day plus PF AT prn in both/12 weeks	CsA Better: Signs: TBUT, meibomian gland expressibility; No difference: OSDI	Signs: TBUT, meibomian gland expressibility, lid inflammation, palpebral conj injection; Symptoms: ODSI	11% burning/ discomfort/ intolerance. Recovered after stopping CsA	[36]
Demiryay *et al.*	Level 2A prospective randomized, investigator-masked study, patients unmasked to therapy	42	Patients with objective dry eye (SchII <10, TBUT <10)	CsA 0.05% emulsion + PF AT vs PF AT alone (Tears Naturale Free, Alcon, TX, USA)/4 months)	CsA Better: Signs: TBUT, SchI, corneal/conj staining and goblet cell density. No difference: none	Signs: Significance from baseline was not determined	None	[32]
Rao *et al.*	Level 2A single-center, investigator-masked, prospective, randomized, longitudinal trial	58	Patients with dry eye symptoms (no specific cut-offs)	CsA 0.05% emulsion vs AT 2× day (Refresh Endura) plus AT prn in both/at least 12 months	CsA Better: Signs: Sch, TBUT, corneal staining, goblet cell density. Symptoms: ODSI; No difference: none	More disease progression in AT group vs CsA group	Discomfort	[34]
Kim *et al.*	Level 3 randomized, prospective, multicenter, non-masked study	150	Patients with dry eye symptoms (OSDI ≥25, SchI <5, TBUT <5; PEE ≥1)	CsA 0.05% emulsion vs vitamin A (Viva, SD, USA) 4× day vs none plus PF AT in all groups (Refresh Plus, Allergan/3 months	CsA Better: Signs: Sch, corneal staining, TBUT, IC, Goblet cell density (0.05% CsA and Vitamin A group vs control); Symptoms: blurry vision (0.05% CsA and Vitamin A vs control) No difference: Photophobia, Irritation	Symptoms: Photophobia (all groups including control), irritation (0.05% CsA and Vitamin A vs control)	No information	[33]

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Table 1. Summary of studies using cyclosporine for the treatment of moderate dry eye (cont.).

Study	Evidence level	N	Population	Dose/treatment length	Efficacy		Side effects	Ref.
					CsA versus control	CsA versus baseline†		
Roberts *et al.*	Level 3 single-center, prospective, randomized, non-masked clinical trial	30	Patients with dry eye symptoms (PEE ≥2)	CsA 0.05% emulsion vs punctal occlusion (Parasol, Odyssey Medical, TN, USA) vs combination [CsA + plug] plus AT prn in all/6 months	CsA Better: Symptoms: AT use (CsA + plug) No difference: staining	Signs: Schl (CsA + plug and CsA), staining (CsA + plug and CsA); Symptoms: AT use (CsA)	One in CsA group withdrew for burning	[37]
Byun *et al.*	Level 4 non-randomized, prospective, multicenter, open-label, surveillance study	362	Patients with dry eye symptoms (no specific cut-offs)	CsA 0.05% emulsion plus AT prn (no control group)/3 months	No control	Signs: Sch, conj staining; Symptoms: burning/stinging, itching, sandy/gritty, blurred vision, photophobia	Ocular pain (11%); irritation (6%)	[31]
Hardten *et al.*	Level 4 single-center, non-randomized, open-label, prospective clinical trial	19	Patients with dry eye (code 375.15) and CsA and AT ≥3 months	CsA 0.05% emulsion + CMC 0.5% (Optive) for 3 months	No control	Signs: TBUT, conj staining; Symptoms: OSDI, discomfort	No adverse events reported	[38]
Perry *et al.*	Level 4 non-randomized, open-label, prospective clinical study	143	Patients with mild, moderate and severe dry eye symptoms (OSDI ≥25)	CsA 0.05% emulsion plus AT/LH prn, plugs allowed/at least 3 months	No control	Signs: TBUT (all), Schirmer (moderate and severe), staining (all), most improved in severe; Symptoms: OSDI (all), most improved in mild.	Seven did not complete 3 months due to burning	[39]
Sahli *et al.*	Level 4 single-center, non-randomized, no control, 6-month, prospective study	45	Patients with objective dry eye (Sch <5)	CsA 0.05% emulsion plus AT 5× day/6 months	No control	Signs: Schl (all populations), TBUT (not SS population)	No information	[41]

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Table 1. Summary of studies using cyclosporine for the treatment of moderate dry eye (cont.).

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					CsA versus control	CsA versus baseline†		
Toker *et al.*	Level 4 single-center, non-randomized, non-masked, no control, prospective study	37	Patients with dry eye symptoms (Sch <10, TBUT <7, PEE)	CsA 0.05% emulsion plus PF AT prn/6 months	No control	Signs: corneal and conjunctival sensitivity, TBUT, Sch, Schl, corneal staining; Symptoms: OSDI (months 6)	No information	[42]
Wang *et al.*	Level 4 single-center, non-randomized, non-masked, no control, prospective study	14	Patients with dry eye symptoms and (SchII ≤5, PEE ≥1, TBUT ≤10)	CsA 0.05% emulsion/2 months	No control	Signs: upper and lower tear meniscus height and volume	No information	[50]
Yuksel *et al.*	Level 4 single-center, non-randomized, non-masked, no control, prospective study	40	Patients with dry eye symptoms (OSDI >10) and (SchI <5, PEE >1, TBUT <10).	CsA 0.05% emulsion, AT × 1 month/ 24 weeks	No control	Signs: SchI, TBUT, goblet cell density; Symptoms: OSDI	No information	[49]
Stonecipher *et al.*	Level 4 single-center, non-randomized, non-masked, no control	5884	Patients with dry eye diagnosed by provider	CsA 0.05% emulsion/ AT varied/2 months	No control	Symptoms: improved symptoms and decreased activity impairments, >60% decreased AT use	No information	[40]

The studies have been cited in order of level of evidence. Each study has been categorized from Level 1 through 4 as follows: Level 1A – a randomized, double-masked design; Level 1B – a randomized, double-masked design, with weak patient masking due to significant variations in treatment versus placebo side-effect profiles; Level 2A – randomized‡, single-masked design; Level 2B – a randomized, single-masked design, with weak masking due to significant variations in treatment versus placebo side-effect profiles; Level 3 – randomized, non-masked; Level 4 – non-randomized, non-masked.
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Baiza-Durán *et al.*	Level 1A multicenter, randomized, double-masked, vehicle-controlled, clinical trial	183	Patients with symptomatic ATD (one eye: SII <5, PEE >3 [of 15])	Aqueous CsA (0.1%, 0.05%) vs aqueous placebo vehicle plus BAK preserved methylcelluluose 0.5% × 8 day/98 days	CsA Better: Symptoms: Red eye & ocular fatigue (0.1% better than 0.05%, control), dryness (0.1% better than 0.05%), photophobia, Ocular fatigue (0.05% better than vehicle); No difference: all signs. Tearing, foreign body sensation.	Signs: TBUT (0.1%), corneal surface (0.05%), Sch and staining (all CsA); Symptoms: dryness (0.1%), photophobia (0.05%), tearing and foreign body sensation (all)	No serious events	[43]
Moon *et al.*	Level 2A single-center, non-randomized‡, single-masked, prospective study	36	Patients with objective dry eye (SchI ≤5 or TBUT <6)	Aqueous CsA 0.05% 4× day one eye vs 0.08% chondroitin sulfate and 0.06% sodium hyaluronate other eye 4× day/6–8 weeks	CsA Better: Signs: TBUT, Goblet Cell density No difference: SchI, epithelial morphologies, nucleus to cytoplasmic ratio	Signs: SchI, epithelial morphologies, nucleus to cytoplasmic ratio	No information	[47]

The studies have been cited in order of level of evidence. Each study has been categorized from Level 1 through 4 as follows: Level 1A – a randomized, double-masked design; Level 1B – a randomized, double-masked design, with weak patient masking due to significant variations in treatment versus placebo side-effect profiles; Level 2A – randomized‡, single-masked design; Level 2B – a randomized, single-masked design, with weak masking due to significant variations in treatment versus placebo side-effect profiles; Level 3 – randomized, non-masked; Level 4 – non-randomized, non-masked.
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Human studies of CsA for dry eye

CsA for the treatment of moderate–severe dry eye

Many studies reported a positive effect of CsA on the symptoms and signs of dry eye in patients with moderate to severe disease (Table 1) [25,30–39]. It is difficult to directly compare these studies as many used different inclusion criteria in their definition of dry eye and different primary outcome measures. In addition, some studies included a control group [25,30,34–36], while others did not [31,39–42]. In general, however, symptom scores improved, ocular staining decreased and Schirmer scores increased in most studies after treatment with CsA [25,30–34,40,43–45]. In a survey study completed by 5884 patients, one-third of patients reported decreased symptom severity by 1 week and two-thirds by 3 weeks [40]. Other factors that have been found to improve with CsA treatment are goblet cell density [32–33,41,46–49], corneal sensitivity [42], and tear meniscus height and volume [50].

When compared with vehicle controls and/or active treatments, improvements in objective and subjective metrics were less consistent. CsA emulsion showed almost uniform superiority to vehicles in improving two objective signs, corneal staining [25,30,44] and Schirmer scores [25,30,35,44], in randomized vehicle-controlled trials, definitively outperforming vehicle in three of the four studies using emulsion placebo [25,30,44]. However, in the only aqueous-based study, aqueous CsA was superior to aqueous vehicle with respect to symptoms only [43]. In all vehicle studies, CsA was superior to vehicle control with respect to at least one additional symptom and/or sign. While the most common symptom to improve was ocular dryness, on average the particular symptom and/or sign that improved was not consistent between studies. For example, Chen *et al.* found that CsA emulsion was superior to vehicle with regards to ocular dryness, total dry eye score, foreign body sensation, Schirmer scoring and corneal staining but not for specific symptoms like photophobia and burning nor for specific signs like TBUT [30]. In partial contrast, the aqueous-based study by Baiza-Duran *et al.* reported that aqueous CsA was superior to vehicle with regards to ocular dryness, photophobia and ocular fatigue but not for other symptoms, like tearing or foreign-body sensation [43]. While both Chen *et al.* and Baiza-Duran *et al.* saw improvements in ocular dryness, Sall *et al.* found that CsA emulsion was superior to vehicle in improving the symptom of blurry vison only [25]. But like Chen *et al.*, Sall *et al.* improved the signs of corneal staining and Schirmer scoring over vehicle [25]. Stevenson *et al.* compared various CsA emulsion concentrations (0.05, 0.1, 0.2, 0.4% CsA) to vehicle and found the highest CsA concentration studied, 0.4% CsA, to have superior Schirmer scores with respect to vehicle and 0.2% CsA [35]. While the 0.1% CsA was shown to have superior conjunctival staining compared with vehicle, 0.4% CsA and 0.05% CsA [35]. In this study, all CsA concentrations compared with vehicle were superior with respect to sandy/gritty sensation and most (0.05, 0.02, 0.4%) were superior with respect to ocular dryness [35]. The inconsistencies in symptom improvement between studies may be partially explained by the positive action of the vehicle in the control group and/or the irritative nature of CsA in the treatment group [25].

With regards to CsA's efficacy over other dry eye treatments, CsA emulsion was found to have a similar efficacy profile to topical vitamin A in one dry eye population [33]. In another population comparing aqueous-based CsA suspensions to aqueous vehicles, CsA was found to have enhanced symptomatic improvement with no significant differences in objective findings [43].

Regarding ADDE versus EDE, two Level 1 studies primarily treated ADDE [25,35] and one primarily treated EDE [36] with CsA. In the two ADDE studies, Schirmer scores and staining (corneal [25] and conjunctival [35]) significantly improved over control, but no difference was seen with regards to TBUT [25,35]. Conversely, in the EDE study, TBUT improved but not staining or Schirmer scores [36].

CsA for the treatment of severe dry eye

CsA's efficacy has been evaluated in the most severe dry eye conditions including Sjogren's syndrome [51], trachoma [44], radiation-associated [52], graft versus host disease [50,53] and Stevens–Johnson Syndrome [45]. It is notable that the most devastating forms of severe dry eye are encountered in trachoma, radiation injury and graft versus host disease. These are more severe in terms of inflammatory signs, dry eye signs and symptoms, as well as more complex in etiology, including primary damage to the lacrimal gland, meibomian gland, cornea and conjunctiva, often with features that would be irreversible by any means of treatment [44]. Thus, it can be challenging to compare the results in severe dry eye. CsA emulsion worked well in the setting of Sjogren's syndrome [51] and trachoma [44], but less well in radiation-associated dry eye [52]. More frequent dosing or a higher concentration of CsA (i.e., 0.1 vs 0.05%) may be options for such patients [43,54], although there are no data that show a benefit to substantially higher concentrations (i.e., 1–2% CsA) compared with lower doses of CsA (0.05–0.1% CsA) in these patients. Dastjerdi *et al.* found that in 22 patients with severe dry eye whose symptoms failed to improve after twice-daily dosing, 15 (68%) noted improvement with more frequent dosing (three- or

four-times daily) [54]. Likewise, in 183 patients 0.1% aqueous CsA was associated with significant improvement in red eye, dryness, photophobia and ocular fatigue when compared with 0.05% aqueous CsA [43]. Other studies, however, did not find a dose effect with CsA emulsion [35]. In eyes where the onset of dry eye can be anticipated (e.g., after allogeneic bone marrow transplant), pretreatment is an option and, in fact, CsA emulsion has been retrospectively shown to decrease dry eye symptoms compared with delayed treatment (Table 2) [53].

CsA for the treatment of post-refractive surgery & contact lens associated dry eye

Two studies have evaluated the effect of CsA emulsion after refractive surgery. A retrospective study of 40 patients found that symptoms and TBUT returned to baseline faster in CsA emulsion-treated eyes (4 vs 8 weeks), but did not find differences in refractive outcomes [55]. On the other hand, a prospective study of 21 patients found no significant differences in dry eye symptoms or tear parameters, but slightly better refractive outcomes in the CsA emulsion-treated group (higher frequency of patients within 0.5 D (Diopters) of intended correction at 3 months) [56]. In contact lens associated dry eye, one prospective study found a positive effect of CsA emulsion with improved symptoms and temporal conjunctival staining [57], while another found no effect of CsA over artificial tears (Table 3) [58].

Long-term effects of CsA emulsion 0.05% for the treatment of dry eye

The long-term effects of CsA are less well studied. One study found that in patients treated with CsA emulsion for 1 year, substituting artificial tears led to worsening dry eye symptoms and signs, suggesting the need for maintenance therapy [59]. Decreasing the frequency of administration may be an option; for example, one study demonstrated maintenance of effect with once-daily dosing after stable treatment with twice-daily dosing for 1 year [60]. A small subset of the population, however, may achieve long-term resolution of chronic dry eye symptoms after long-term CsA emulsion use. This was reported to occur in eight patients (4% of CsA treated dry eye population) who remained symptom and sign free for a mean of 21 months (range 16–29) after using CsA emulsion for 6–72 months [61]. An exciting question that has not yet been definitively answered is whether early treatment can prevent progression to a more severe dry eye phenotype. Several studies had suggested that this may be the case. Rao *et al.* reported that more patients progressed to a more severe disease stage when treated with artificial tears versus CsA emulsion (7 of 22 vs 2 of 36, $p < 0.01$) [34]. Another study involving treatment in patients before autoimmune manifestations from an allogeneic bone marrow transplant noted a decrease in symptom severity as well [53].

CsA at higher doses for the treatment of dry eye

In general, studies looking at 0.1% CsA solutions found them to be safe for long-term use [62] and superior to vehicle [43,62]. The most effective formulations of CsA for continued research are shown to be the 0.05 and 0.1% doses [25,35,43]. However, there are discrepancies between studies on whether 0.1% dose is better than the 0.05% one. Studies in favor of the 0.1% dose included a randomized, controlled study using aqueous CsA that found the 0.1% to be superior to the 0.05% and aqueous control in treating red eye and ocular fatigue. In addition, the 0.1% was also superior to the 0.05% in treating dryness [43]. Studies in favor of the 0.05% dose include a randomized controlled study using CsA emulsion which found the 0.05% CsA formulation to be significantly better than the 0.1% or control in improving blurry vision symptoms [25]. However, some studies have found no differences between these doses including a randomized, vehicle controlled study using various CsA emulsion concentrations (0.05, 0.1, 0.2, 0.4%). However, they did report that 0.1% CsA performed most consistently overall while 0.05% CsA showed the most consistent symptom improvement [35]. Similarly, a nonrandomized open-label study found no statistical difference between the two 0.05 and 0.1% emulsion concentrations with respect to objective (fluorescein staining, Schirmer scoring) and subjective measures (survey) [62].

Studies evaluating the safety and efficacy of 2% CsA emulsion have mostly focused on patients with more severe dry eye. In patients with acquired primary lacrimal disease, this treatment improved subjective symptoms (grittiness) and Schirmer scores compared with placebo [63]. However, in Sjogren's patients from the same study, no significant improvement in subjective symptoms or Schirmer scoring was seen. Conversely, another study using 2% CsA emulsion in Sjogren's syndrome did show significant improvement in TBUT and Rose Bengal scoring when compared with placebo [23]. As there are no direct studies comparing the 2% formulation with that of lower doses, it is not possible to conclude whether the higher dose portends extra benefit in patients with dry eye.

CsA & its effect on subclinical markers on the ocular surface

Studies have found improved metrics of ocular surface health after CsA treatment, including decreased

Table 2. Summary of studies using cyclosporine for the treatment of severe dry eye.

Study	Evidence level	N	Population	Dose/treatment length	Efficacy		Side effects	Ref.
					CsA versus control	CsA versus baseline†		
Deveci *et al.*	Level 3 single-center, randomized, control group, non-masked, prospective study	46	Patients with SS dry eye (no specific cut-offs)	CsA 0.05% emulsion vs AT/1 month	CsA better: Signs: SchI, TBUT, red eye; Symptoms: burning, photophobia, pain; No difference: none	Improved in all	None	[51]
Guzey *et al.*	Level 3 single-center, randomized, control group, masking unclear, prospective study	64	Patients with trachoma (OSDI >22, PEE ≥4, SchII ≤5, TBUT ≤5)	CsA 0.05% emulsion vs emulsion placebo vehicle plus AT 5 x day (Tears Naturale Free, Alcon, France)/6 months	CsA better: Signs: corneal staining, Sch, IC, goblet cell density, TBUT. Symptoms: all subjective symptoms, OSDI; No difference: none	Improved in all	Burning, stinging 16% but no withdrawal	[44]
Malta *et al.*	Level 3 retrospective, comparative, non-masked, interventional case series with control group	155	Patients with GVHD	CsA 0.05% emulsion started 1 month prior BMT vs CsA ≥6 months after BMT	CsA better: Signs: SchI, TBUT; No difference: corneal and conjunctival staining, OSDI	Dry eye symptoms more severe in groups with delayed CsA treatment. Significance from baseline was not determined.	No information	[53]
Hoehn *et al.*	Level 4 retrospective review of clinical records with no control group, non-masked	11	Children with radiation associated dry eye	CsA 0.05% emulsion plus AT (no control group)/6 months	No control	Dry eye symptoms and signs partially improved in three patients	All transient burning/ irritation	[52]
Prabhasawat *et al.*	Level 4 prospective non-comparative interventional case series with no control group	30	Patients with SJS associated dry eye (SchI ≤5, PEE)	CsA 0.05% emulsion plus PF AT (no control group)/6 months	No control	Signs: corneal staining, SchI. Symptoms: foreign body sensation, photophobia, pain, dryness, conj. injection	7/30 discontinued CsA pain, injection, swelling, dryness	[45]

The studies have been cited in order of level of evidence. Each study has been categorized from Level 1 through 4 as follows: Level 1A – a randomized, double-masked design; Level 1B – a randomized, double-masked design, with weak patient masking due to significant variations in treatment vs placebo side-effect profiles; Level 2A – randomized, single-masked design; Level 2B – a randomized, single-masked design, with weak masking due to significant variations in treatment vs placebo side-effect profiles; Level 3 – randomized, non-masked; Level 4 – non-randomized, non-masked.
CsA dosed twice a day unless otherwise noted.
†Signs and symptoms listed where CsA group had improved parameters compared with baseline but not compare to control.
AT: Artificial tears; BMT: Bone marrow transplant; CsA: Cyclosporine; GVHD: Graft versus host disease; IC: Impression cytology; OSDI: Ocular surface disease index; PEE: Punctate epithelial erosions; PF: Preservative free; Sch: Schirmer; SchI: Schirmer's without anesthesia; SchII: Schirmer's with anesthesia; SJS: Stevens–Johnson Syndrome; SS: Sjogren's syndrome; TBUT: Tear break up time.

Table 3. Summary of studies using cyclosporine for the treatment of post-refractive surgery and contact lens associated dry eye.

Study	Evidence level	N	Population	Dose/treatment length	Efficacy		Side effects	Ref.
					CsA versus control	CsA versus baseline†		
Salib *et al.*	Level 1B randomized, parallel, double-masked, prospective clinical trial	21	Patients with dry eye undergoing myopic LASIK	CsA 0.05% emulsion vs AT/3 months	CsA Better: eyes within 0.5 D of target spherical equivalent; No difference: Sch, superficial punctate keratitis, uncorrected visual acuity, OSDI	Signs: Sch, uncorrected visual acuity	No differences between groups	[56]
Willen *et al.*	Level 1B randomized, double-masked, placebo-controlled, prospective study	44	Patients with contact lens associated dry eye	CsA 0.05% emulsion vs PF AT/3 months	No difference between groups	No statistically significant improvement mentioned	No information	[58]
Hom *et al.*	Level 2A randomized, investigator-masked, prospective, placebo-controlled clinical trial	17	Patients with contact lens associated dry eye	CsA 0.05% emulsion vs AT CMC 0.5% (Refresh)/5 weeks	CsA Better: Signs: temporal conjunctival staining; Symptoms: AT use, dryness; No difference: corneal staining, bulbar conjunctival staining, TBUT, OSDI, visual acuity, biomicroscopy	Symptoms: contact wearing time	No differences between groups	[57]
Lee *et al.*	Level 4 retrospective, nonrandomized, comparative analysis with control group	40	Patients undergoing LASEK	CsA 0.05% emulsion + AT vs AT/8 weeks	CsA Better: Signs: TBUT(at 4 wks) Symptoms: lower symptoms scores (at 4 wks); No difference: refraction, Sch, staining	Signs: TBUT; Symptoms: lower symptoms scores (1 and 2 wks)	None	[55]

The studies have been cited in order of level of evidence. Each study has been categorized from Level 1 through 4 as follows: Level 1 – a randomized, double-masked design; Level 2 – randomized, single-masked design; Level 3 – randomized, non-masked; Level 4 – non-randomized, non-masked.
CsA dosed twice a day unless otherwise noted.
†Signs and symptoms listed where CsA group had improved parameters compared with baseline but not compare to control.
AT: Artificial tears; CMC: Carboxymethylcellulose; CsA: Cyclosporine; LASEK: Laser epithelial keratomileusis; LASIK: Laser *in situ* keratomileusis; PF: Preservative free; Sch: Schirmers.

inflammatory marker expression (HLA-DR, CD40L, CD11a, IL-6, IL-8) [46,64–65], decreased inflammatory cell levels (HLA-DR positive, Fas positive, CD11a positive, CD3 positive cells, TGF-2 positive goblet cells) [46,64] and increased mucin production [66]. For example, in 44 patients treated with 0.05% CsA emulsion for 3 months (21 with and 23 without initial methylprednisolone), tear IL-6 and -8 levels were reduced at 3 months compared with baseline [67]. Interestingly, when comparing two doses of CsA emulsion (0.05 vs 0.1%), the lower dose outperformed the higher with respect to time and magnitude of inflammatory marker reduction [64,65]; furthermore, only 0.05% CsA significantly decreased the percentage of Fas-positive cells [64]. In 13 eyes of chronic graft versus host disease associated dry eye, inflammatory cell numbers were decreased in the 0.05% CsA emulsion treated group (four-times a day) compared with the control group [50].

Regarding mucin production, in goblet cell monolayers treated with 1-μM CsA emulsion, a significantly higher percentage of mucin-filled secretory granules and mucin volume was seen, averaging 194% of the control level [66]. Likewise, in six dry eye patients, an increased number of TGF-β2 (an immunoregulator) positive goblet cells were noted after 6–12 weeks of CsA emulsion [48].

Cyclosporin & its role in the reflex control of tear homeostasis through inflammatory modulation in dry eye

The improvement seen in corneal staining with CsA use may indicate a broader effect as the ocular surface, lacrimal glands and the neuronal feedback loop that link them effectively make a single sensory apparatus for ocular surface homeostasis [25]. Sensory information sent via the sensory and autonomic pathways can influence tear production and composition [25]. Several studies suggest that decreases in inflammation and improvements in ocular epithelial surface may result in better stimulation of the nerve endings in the cornea and conjunctiva by blinking [25,56,65]. Indeed, an animal study evaluating the lacrimomimetic effect of CsA emulsion a few hours before and after administration found a significant increase in both tear production (lacrimal gland fluid flow rate and Schirmer) and blink rate compared with vehicle [28]. Through its ability to modulate inflammation and improve the ocular epithelium, CsA may play a role normalizing neural signals to the lacrimal gland, in turn, improving the quantity and quality of tear production [25]. This hypothesis is supported by improvements in Schirmer values after CsA treatment seen in most randomized vehicle controlled studies [25,30,35,43–44].

CsA & its effect on the ocular microbiome

No ocular infections have been reported in any of the CsA trials. In fact, patients treated with CsA were generally found to have fewer microbes on their ocular surface (24 of 47 patients positive) than vehicle-treated patients (9 of 11 patients positive) [35]. There was also a trend for a decrease in bacterial species and total strains of organisms after 12 weeks of CsA treatment versus an increase in these parameters after vehicle treatment. Overall, changes in flora were noted in all dry eye patients over a 12-week period, independent of treatment [35]. Due to the immune modulating effects of Restasis, many healthcare professionals avoid its use in patients with a history of herpes keratitis; however, its use in such patient populations has never been studied.

Side effects of CsA for dry eye

The main side effect of CsA 0.05% emulsion is ocular surface pain (described using various terminology including aching, burning) and irritation and this is acknowledged in the Restasis package insert (ocular burning, 17%) [22]. Other studies have also reported pain as the drug's most frequent side effect [31]. In a study of 35 dry eye patients who discontinued CsA after less than 12 weeks of use, burning was found to be the reason for discontinuation in 60% of individuals [68]. Likewise, in a prospective study of CsA for Stevens–Johnson Syndrome, 8 of 30 withdrew from the study as a result of adverse symptoms (pain, redness and eyelid swelling) [45].

The use of loteprednol etabonate 0.5% (Lotemax; Bausch & Lomb) both pre-CsA and with initiation of therapy was shown to reduce the frequency of severe stinging [69]. A similar finding was reported with 1% methylprednisolone [67]. In 21 subjects treated with combination 1% methylprednisolone and CsA for 3 weeks followed by CsA alone, symptoms, Schirmer scores and corneal staining scores were better at 1 month compared with a group (n = 23) started on CsA alone [67].

However, patients who initially discontinue CsA can be re-challenged as demonstrated by the Physician's Evaluation of Restasis Satisfaction In Second Trial (PERSIST) study [31]. In this retrospective review, a second CsA trial was attempted in 35 patients who discontinued the medication after less than 12 weeks of use. In the second trial, physician education was given in 97% of cases and topical corticosteroids in 29%. Per physician report, 80% of patients achieved a clinical benefit on this second trial [68].

Studies have evaluated the effect of CsA on corneal morphologic and functional properties and

have reported no change in thickness (by ultrasound pachymetry), endothelial cell density (by specular microscopy), topography (by Orbscan II), or corneal biomechanics (by Ocular Response Analyzer) with treatment [70,71].

Comprehensive review of CsA & its use in dry eye

To summarize data across studies, CsA is no doubt an effective treatment for dry eye in some patients and its performance is at or above the level of the control. Its utility is limited, however, by its side-effect profile (ocular pain) and the fact that artificial tears (the control) also decrease symptoms and signs of dry eye [32]. The evidence supports that CsA emulsion improves specific objective findings of dry eye (staining, Schirmer) better than emulsion vehicle [25,30,35,44] and most artificial tear studies [32–34]. Dry eye symptom improvement has been more variable, with the most consistent improvement seen with respect to the complaint of ocular dryness [30,35,57]. Other specific situations where CsA may be particularly helpful is in the bone marrow transplant population, where the 'time-zero' of dry eye development is known and where CsA may be used as a preventative treatment [34].

Translational prospects

Due to the highly lipophilic and poorly water soluble nature of CsA, it must be formulated as oil-based preparations. Unfortunately, these solutions are poorly tolerated and have a low bioavailability because CsA is nonpolar and has a greater attraction to the lipophilic vehicle rather than to the tissue [72]. Development of different delivery mechanisms, such as aqueous preparations, have been explored to provide an increase in corneal drug tissue levels, bioavailability and tolerability. Indeed several studies exist in the literature comparing aqueous vehicles to the gold standard oil-based emulsion, Restasis [72–74].

The two main water-based preparations tested have been different variations of nanoparticle-based suspensions and/or micellar solutions. In a study measuring both tolerability and tissue uptake in rabbit corneas, Luschmann *et al.* evaluated an *in situ* nanosuspension [0.4% CsA] and a micellar solution [0.05% CsA] as delivery systems for CsA [72]. Both solutions were tolerated well, evoking minimal to no irritation. The nanosuspension and micellar solution also delivered high levels of CsA, exceeding drug tissue levels reported for Restasis as well as cationic emulsions [72]. In another study therapeutically active CsA levels were achieved in tissues of both the anterior and posterior segments using a water-soluble CsA prodrug formulated within an aqueous solution. The results also indicated higher bioavailability and lower elimination rate when compared with Restasis [73]. A different micellar solution made and studied by Di Tommaso *et al.* was also shown to have adequate penetration. Using Schirmer testing and osmolarity measurements, they demonstrated no altering effects on ocular surface properties [75]. Another study by Khan *et al.* using nanoparticle suspensions reported that CsA aqueous-based solutions may even be less irritating to the eye than lipophilic emulsions [74]. They found their aqueous suspension caused less local irritation with nearly the same penetrability of CsA compared directly to Restasis [74]. Their success was believed to be attributed to the use of triglycerides in the nanosuspension to replace the high concentrations of surfactants, a known eye irritant present in Restasis.

Other novel delivery systems have been developed for CsA delivery including contact lenses, punctal plugs and implants [76]. Pioneering studies looked at the exploration of CsA delivery from contact lenses to provide controlled and extended drug delivery with an increased bioavailability when treating either chronic dry eyes or contact lens mediated dry eyes. The 1-DAY ACUVUE® lens releases CsA for about a day and an extended wear silicone hydrogel (SiH) lens releases CsA for about 2 weeks [76]. A compound of vitamin E and CsA has been synthesized with a more favorable partition coefficient, favoring bioavailability. The increased partition coefficient has allowed researchers to increase the duration of CsA release in both types of contacts, with the hopes of creating a 1-month CsA release SiH lens [76].

A punctal plug has been developed, designed to release CsA over a 3-month period and provide dual mechanisms of dry eye treatment. Implants have also been explored as a drug delivery mechanism. Kim *et al.* explored the use of episcleral CsA implants to deliver CsA to the lacrimal gland and showed efficacy in the treatment of canine keratoconjunctitis sicca. It was proposed that this approach might reduce the level of lacrimal gland pathology associated with human graft versus host disease after allogeneic hematopoietic stem cell transplantation [77]. While none of these novel formulation approaches and devices involved human trials, the results of these early studies suggest that the comfort of CsA may be improved without compromising efficacy.

Conclusion

Dry eye is an important public health problem given its prevalence, morbidity and cost implications. Inflammation has been shown to play a role in dry eye, resulting in a reduced tear production [25,35,56,65]. Several studies suggest that decreased inflammation and an improved epithelial surface may result in improved quantity and quality of tear produc-

tion through normalization of reflex control of tear homeostasis [25,56,65]. Indeed, Restasis outperformed vehicles and artificial tears in most of the randomized controlled trials, consistently decreasing corneal staining [25,30,32–34,44] and increasing Schirmer scores [25,30,32–35,44]. CsA also decreased ocular surface inflammation measured by T lymphocyte activation [25] and tear levels of inflammatory mediators [27,64–65].

The effect of CsA on dry eye symptoms has been less impressive. Overall, Restasis showed significant improvement compared with vehicle with respect to at least one symptom, typically ocular dryness [30,35,57], with inconsistencies in improvements in symptoms between studies [25,30,35,43–44]. Likely reasons behind the limited symptom improvement of CsA emulsion compared with vehicle are the beneficial effects of the vehicle and the side effects of Restasis. In the future, new CsA formulations, including aqueous-based nanoparticle suspensions and micellar solutions, will likely allow for products with improved tolerability without compromised efficacy [72–74].

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Executive summary

Symptoms & signs of dry eye

- Symptoms of dry eye were assessed using dry eye surveys specific to each study. Objective signs were measured using tear breakup time, corneal staining, Schirmer scoring, and morphologic and qualitative characterization of the eyelid margin and meibomian glands.

Inflammation & dry eye

- In animal models T-cell-mediated inflammation was both a cause and result of dry eye [12]. In humans, dry eye was found to be associated with conjunctival T cells and elevated levels of inflammatory cytokines (IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-17, TNF-α) in the tears compared with controls [18,20].

Cyclosporin's mechanism of action, basic pharmacology & pharmacokinetics

- Mechanism of action
  - Cyclosporine (CsA) enters T cells and binds cyclophilin. The CsA/cyclophilin complex affects T-cell activity, blocking calcinuerin and preventing NFATc dephosphorylation. The subsequent reduction in IL-2 levels reduces the function of effector T cells.
- Formulation
  - Restasis® is formulated with 0.05% CsA in a homogenous emulsion of glycerin (2.2%), castor oil (1.25%), polysorbate 80 (1.00%), carbomer copolymer type A (0.05%) and purified water (to 100%) [22].
- Blood concentration
  - No quantifiable CsA levels were found in the blood of patients treated with 0.05% CsA [24,25].

Methods

- A PubMed search was conducted using the terms 'CsA dry eye'. All human studies as well as basic science articles evaluating CsA in dry eye were included in this review.

Animal studies of CsA

- In mice, CsA decreased apoptotic cells in the conjunctival epithelium as well as markedly improved ocular and lacrimal gland inflammation [26,27]. In rabbits, CsA decreased $CD4^+$ lacrimal gland lymphocytes [29].

Human studies of CsA for dry eye

- CsA for the treatment of moderate–severe dry eye
  - Mostly, symptom scores improved, ocular staining decreased, Schirmer scores increased and goblet cell density increased after CsA treatment [25,30–34,40,43]. In vehicle-controlled studies there was consistent improvement in the objective signs corneal staining and Schirmer scores [25,30–34,40,43]. Likewise, the subjective symptom ocular dryness was shown to improve with CsA treatment over vehicle in many trials [25,30–34,40,43]. Improvement in other symptoms, however, was more variable.
- CsA for the treatment of severe dry eye
  - CsA was effective in reducing dry eye symptoms and signs in the setting of Sjogren's syndrome and trachoma [44,51]. In addition, one study showed that pretreatment with CsA before dry eye onset in patients undergoing bone marrow transplant decreased dry eye severity [53].

Executive summary (cont.).

- CsA for the treatment of post-refractive surgery & contact lens associated dry eye
  - Studies evaluating CsA use after refractive surgery showed faster symptom improvement and slightly better refractive outcomes [55,56]. In contact lens associated dry eye, CsA improved symptoms and temporal conjunctival staining in one study and had no effect over artificial tears in the other [57,58].
- Long-term effects of CsA emulsion 0.05% for the treatment of dry eye
  - Studies suggest that CsA may need to be used for the long term, although the frequency of maintenance therapy can be decreased [59].
- CsA at higher doses for the treatment of dry eye
  - Studies looking at higher 0.1% CsA solutions found them safe for long-term use. The 0.05 and 0.1% formulations of CsA were found to be the most effective [25,35,43]. Studies of 2% CsA showed significant improvement when compared with placebo [23,63].
- CsA & its effect on subclinical markers on the ocular surface
  - CsA treatment improved subclinical metrics on ocular surface including decreased inflammatory marker expression, decreased inflammatory cell levels and increased mucin production [46,64–65].
- CsA & its effect on the ocular microbiome
  - No ocular infections have been reported in any of the CsA trials [35].
- Comprehensive review of CsA & its use in dry eye
  - After reviewing the literature it is determined that CsA is most applicable to patients with moderate to severe dry eye (Schl <5, TBUT <5, PEE >5), particularly in those complaining of ocular dryness [30,35,43,57].

Side effects of CsA for dry eye

- The major side effect of CsA 0.05% emulsion is ocular surface pain and irritation [22,31].

Future perspective

- Basic research studies show that newer aqueous-based nanoparticle suspensions and micellar solutions can have increased bioavailability and better tolerability compared with the lipophilic delivery system currently used in Restasis [72–74]. Other novel delivery systems have been development for CsA delivery including contact lenses, punctal plugs and implants [72–74].

Conclusion

- Inflammation has been shown to play a role in dry eye, resulting in a reduction in tear production [25,35,56,58]. CsA significantly decreased inflammatory mediators [65] and improved dry eye symptoms and signs in a majority of patients. Restasis outperformed vehicles in the majority of randomized clinical trials, consistently decreasing corneal staining and increasing Schirmer scores. In addition, Restasis improved dry eye symptoms overall and showed significant improvement compared with vehicle with respect to at least one symptom [25,30,35,43–44]. However, Restasis had no consistencies in the specific symptoms it improved.

References

Papers of special note have been highlighted as:
• of interest; •• of considerable interest.

1 The epidemiology of dry eye disease: report of the Epidemiology Subcommittee of the International Dry Eye WorkShop (2007). *Ocul. Surf.* 5(2), 93–107 (2007).

2 Yu J, Asche CV, Fairchild CJ. The economic burden of dry eye disease in the United States: a decision tree analysis. *Cornea* 30(4), 379–387 (2011).

3 Galor A, Zheng DD, Arheart KL *et al.* Dry eye medication use and expenditures: data from the medical expenditure panel survey 2001–2006. *Cornea* 31(12), 1403–1407 (2012).

4 Pouyeh B, Viteri E, Feuer W *et al.* Impact of ocular surface symptoms on quality of life in a United States veterans affairs population. *Am. J. Opthalmol.*, 153(6), 1061–1066 (2012).

5 Rajagopalan K, Abetz L, Mertzanis P *et al.* Comparing the discriminative validity of two generic and one disease-specific health-related quality of life measures in a sample of patients with dry eye. *Value Health* 8(2), 168–174 (2005).

6 Schiffman RM, Christianson MD, Jacobsen G, Hirsch JD, Reis BL. Reliability and validity of the Ocular Surface Disease Index. *Arch. Ophthalmol.* 118(5), 615–621 (2000).

7 Schein OD, Tielsch JM, Munoz B, Bandeen-Roche K, West S. Relation between signs and symptoms of dry eye in the elderly. A population-based perspective. *Ophthalmology* 104(9), 1395–1401 (1997).

8 Galor A, Feuer W, Lee DJ, Florez H, Venincasa VD, Perez VL. Ocular surface parameters in older male veterans. *Invest. Ophthalmol. Vis. Sci.* 54(2), 1426–1433 (2013).

9 Stern ME, Siemasko KF, Gao J, Calonge M, Niederkorn JY, Pflugfelder SC. Evaluation of ocular surface inflammation in the presence of dry eye and allergic conjunctival disease. *Ocul. Surf.* 3(Suppl. 4), s161–s164 (2005).

10 Gao J, Morgan G, Tieu D *et al.* ICAM-1 expression predisposes ocular tissues to immune-based inflammation in dry eye patients and Sjogrens syndrome-like MRL/lpr mice. *Exp. Eye Res.* 78(4), 823–835 (2004).

11 Stern ME, Gao J, Schwalb TA *et al.* Conjunctival T-cell subpopulations in Sjogren's and non-Sjogren's patients with dry eye. *Invest. Ophthalmol. Vis. Sci.* 43(8), 2609–2614 (2002).

12 Niederkorn JY, Stern ME, Pflugfelder SC *et al.* Desiccating stress induces T cell-mediated Sjogren's syndrome-like lacrimal keratoconjunctivitis. *J. Immunol.* 176(7), 3950–3957 (2006).

13 Schaumburg CS, Siemasko KF, De Paiva CS *et al.* Ocular surface APCs are necessary for autoreactive T cell-mediated experimental autoimmune lacrimal keratoconjunctivitis. *J. Immunol.* 187(7), 3653–3662 (2011).

14 Stern ME, Schaumburg CS, Siemasko KF *et al.* Autoantibodies contribute to the immunopathogenesis of experimental dry eye disease. *Invest. Ophthalmol. Vis. Sci.* 53(4), 2062–2075 (2012).

15 De Paiva CS, Chotikavanich S, Pangelinan SB *et al.* IL-17 disrupts corneal barrier following desiccating stress. *Mucosal Immunol.* 2(3), 243–253 (2009).

16 Okanobo A, Chauhan SK, Dastjerdi MH, Kodati S, Dana R. Efficacy of topical blockade of interleukin-1 in experimental dry eye disease. *Am. J. Opthalmol.*, 154(1), 63–71 (2012).

17 Goyal S, Chauhan SK, Zhang Q, Dana R. Amelioration of murine dry eye disease by topical antagonist to chemokine receptor 2. *Arch. Ophthalmol.* 127(7), 882–887 (2009).

18 Massingale ML, Li X, Vallabhajosyula M, Chen D, Wei Y, Asbell PA. Analysis of inflammatory cytokines in the tears of dry eye patients. *Cornea* 28(9), 1023–1027 (2009).

19 Yoon KC, Jeong IY, Park YG, Yang SY. Interleukin-6 and tumor necrosis factor-alpha levels in tears of patients with dry eye syndrome. *Cornea* 26(4), 431–437 (2007).

20 Lee SY, Han SJ, Nam SM *et al.* Analysis of tear cytokines and clinical correlations in sjogren syndrome dry eye patients and non-sjogren syndrome dry eye patients. *Am. J. Opthalmol.*, 156(2), 247–253 e241 (2013).

21 Gao J, Sana R, Calder V *et al.* Mitochondrial permeability transition pore in inflammatory apoptosis of human conjunctival epithelial cells and T cells: effect of cyclosporin A. *Invest. Ophthalmol. Vis. Sci.* 54(7), 4717–4733 (2013).

22 Allergan www.allergan.com/assets/pdf/restasis_pi.pdf

23 Gunduz K, Ozdemir O. Topical cyclosporin treatment of keratoconjunctivitis sicca in secondary Sjogren's syndrome. *Acta Ophthalmol.* 72(4), 438–442 (1994).

24 Small DS, Acheampong A, Reis B *et al.* Blood concentrations of cyclosporin a during long-term treatment with cyclosporin a ophthalmic emulsions in patients with moderate to severe dry eye disease. *J. Ocul. Pharmacol. Ther.* 18(5), 411–418 (2002)

25 Sall K, Stevenson OD, Mundorf TK, Reis BL. Two multicenter, randomized studies of the efficacy and safety of cyclosporine ophthalmic emulsion in moderate to severe dry eye disease. CsA Phase 3 study group. *Ophthalmology* 107(4), 631–639 (2000).

•• **This was by far the largest and most powerful randomized double-blind vehicle-controlled study and it looked at varying concentrations of Cyclosporine (CsA). Its data are of the greatest value when evaluating CsA treatment.**

26 Jabs DA, Lee B, Burek CL, Saboori AM, Prendergast RA. Cyclosporine therapy suppresses ocular and lacrimal gland disease in MRL/Mp-lpr/lpr mice. *Invest. Ophthalmol. Vis. Sci.* 37(2), 377–383 (1996).

27 Strong B, Farley W, Stern ME, Pflugfelder SC. Topical cyclosporine inhibits conjunctival epithelial apoptosis in experimental murine keratoconjunctivitis sicca. *Cornea* 24(1), 80–85 (2005).

28 Toshida H, Nguyen DH, Beuerman RW, Murakami A. Neurologic evaluation of acute lacrimomimetic effect of cyclosporine in an experimental rabbit dry eye model. *Invest. Ophthalmol. Vis. Sci.* 50(6), 2736–2741 (2009).

29 Thomas PB, Samant DM, Zhu Z *et al.* Long-term topical cyclosporine treatment improves tear production and reduces keratoconjunctivitis in rabbits with induced autoimmune dacryoadenitis. *J. Ocul. Pharmacol. Ther.* 25(3), 285–292 (2009).

30 Chen M, Gong L, Sun X *et al.* A comparison of cyclosporine 0.05% ophthalmic emulsion versus vehicle in Chinese patients with moderate to severe dry eye disease: an eight-week, multicenter, randomized, double-blind, parallel-group trial. *J. Ocul. Pharmacol. Ther.* 26(4), 361–366 (2010).

•• **This was the second largest and most powerful randomized double-blind vehicle-controlled study and thus its data are of immense value when evaluating CsA treatment.**

31 Byun YS, Rho CR, Cho K, Choi JA, Na KS, Joo CK. Cyclosporine 0.05% ophthalmic emulsion for dry eye in Korea: a prospective, multicenter, open-label, surveillance study. *Korean J. Ophthalmol.* 25(6), 369–374 (2011).

32 Demiryay E, Yaylali V, Cetin EN, Yildirim C. Effects of topical cyclosporine a plus artificial tears versus artificial tears treatment on conjunctival goblet cell density in dysfunctional tear syndrome. *Eye Contact Lens* 37(5), 312–315 (2011).

33 Kim EC, Choi JS, Joo CK. A comparison of vitamin a and cyclosporine a 0.05% eye drops for treatment of dry eye syndrome. *Am. J. Opthalmol.*, 147(2), 206–213 (2009).

34 Rao SN. Topical cyclosporine 0.05% for the prevention of dry eye disease progression. *J. Ocul. Pharmacol. Ther.* 26(2), 157–164 (2010).

35 Stevenson D, Tauber J, Reis BL. Efficacy and safety of cyclosporin A ophthalmic emulsion in the treatment of moderate-to-severe dry eye disease: a dose-ranging, randomized trial. The Cyclosporin A Phase 2 Study Group. *Ophthalmology* 107(5), 967–974 (2000).

• **This was a large randomized double-blind vehicle-controlled study that looked at many varying concentrations of CsA including concentrations that no other randomized double-blind vehicle-controlled studies tested. It evaluated both the safety and efficacy of CsA.**

36 Prabhasawat P, Tesavibul N, Mahawong W. A randomized double-masked study of 0.05% cyclosporine ophthalmic emulsion in the treatment of meibomian gland dysfunction. *Cornea* 31(12), 1386–1393 (2012).

37 Roberts CW, Carniglia PE, Brazzo BG. Comparison of topical cyclosporine, punctal occlusion, and a combination for the treatment of dry eye. *Cornea* 26(7), 805–809 (2007).

38 Hardten DR, Brown MJ, Pham-Vang S. Evaluation of an isotonic tear in combination with topical cyclosporine for the treatment of ocular surface disease. *Curr. Med. Res. Opin.* 23(9), 2083–2091 (2007).

39 Perry HD, Solomon R, Donnenfeld ED *et al.* Evaluation of topical cyclosporine for the treatment of dry eye disease. *Arch. Ophthalmol.* 126(8), 1046–1050 (2008).

40 Stonecipher K, Perry HD, Gross RH, Kerney DL. The impact of topical cyclosporine A emulsion 0.05% on the

outcomes of patients with keratoconjunctivitis sicca. *Curr. Med. Res. Opin.* 21(7), 1057–1063 (2005).

41 Sahli E, Hosal BM, Zilelioglu G, Gulbahce R, Ustun H. The effect of topical cyclosporine A on clinical findings and cytological grade of the disease in patients with dry eye. *Cornea* 29(12), 1412–1416 (2010).

42 Toker E, Asfuroglu E. Corneal and conjunctival sensitivity in patients with dry eye: the effect of topical cyclosporine therapy. *Cornea* 29(2), 133–140 (2010).

43 Baiza-Duran L, Medrano-Palafox J, Hernandez-Quintela E, Lozano-Alcazar J, Alaniz-de la OJ. A comparative clinical trial of the efficacy of two different aqueous solutions of cyclosporine for the treatment of moderate-to-severe dry eye syndrome. *Br. J. Ophthalmol.*, 94(10), 1312–1315 (2010).

- **This was a large randomized double-blind vehicle-controlled clinical trial that looked at aqueous vehicles rather than emulsions as well as varying concentrations of CsA.**

44 Guzey M, Karaman SK, Satici A, Ozardali I, Sezer S, Bozkurt O. Efficacy of topical cyclosporine A in the treatment of severe trachomatous dry eye. *Clin. Experiment Ophthalmol.* 37(6), 541–549 (2009).

45 Prabhasawat P, Tesavibul N, Karnchanachetanee C, Kasemson S. Efficacy of cyclosporine 0.05% eye drops in Stevens Johnson syndrome with chronic dry eye. *J. Ocul. Pharmacol. Ther.* 29(3), 372–377 (2013).

46 Kunert KS, Tisdale AS, Gipson IK. Goblet cell numbers and epithelial proliferation in the conjunctiva of patients with dry eye syndrome treated with cyclosporine. *Arch Ophthalmol.* 120(3), 330–337 (2002).

47 Moon JW, Lee HJ, Shin KC, Wee WR, Lee JH, Kim MK. Short term effects of topical cyclosporine and viscoelastic on the ocular surfaces in patients with dry eye. *Korean J. Ophthalmol.* 21(4), 189–194 (2007).

48 Pflugfelder SC, De Paiva CS, Villarreal AL, Stern ME. Effects of sequential artificial tear and cyclosporine emulsion therapy on conjunctival goblet cell density and transforming growth factor-beta2 production. *Cornea* 27(1), 64–69 (2008).

49 Yuksel B, Bozdag B, Acar M, Topaloglu E. Evaluation of the effect of topical cyclosporine A with impression cytology in dry eye patients. *Eur. J. Ophthalmol.* 20(4), 675–679 (2010).

50 Wang Y, Ogawa Y, Dogru M *et al.* Ocular surface and tear functions after topical cyclosporine treatment in dry eye patients with chronic graft-versus-host disease. *Bone Marrow Transplant.* 41(3), 293–302 (2008).

51 Deveci H, Kobak S. The efficacy of topical 0.05 % cyclosporine A in patients with dry eye disease associated with Sjogren's syndrome. *Int. Ophthalmol.* 34(5), 1043–1048 (2014).

52 Hoehn ME, Kelly SR, Wilson MW, Walton RC. Cyclosporine 0.05% ophthalmic emulsion for the treatment of radiation-associated dry eye in children. *Pediatr. Blood Cancer* 60(7), E35–E37 (2013).

53 Malta JB, Soong HK, Shtein RM *et al.* Treatment of ocular graft-versus-host disease with topical cyclosporine 0.05%. *Cornea* 29(12), 1392–1396 (2010).

54 Dastjerdi MH, Hamrah P, Dana R. High-frequency topical cyclosporine 0.05% in the treatment of severe dry eye refractory to twice-daily regimen. *Cornea* 28(10), 1091–1096 (2009).

55 Lee HS, Jang JY, Lee SH, Im SK, Yoon KC. Clinical effectiveness of topical cyclosporine a 0.05% after laser epithelial keratomileusis. *Cornea* 32(7), e150–e155 (2013).

56 Salib GM, McDonald MB, Smolek M. Safety and efficacy of cyclosporine 0.05% drops versus unpreserved artificial tears in dry-eye patients having laser in situ keratomileusis. *J. Cataract Refract. Surg.* 32(5), 772–778 (2006).

57 Hom MM. Use of cyclosporine 0.05% ophthalmic emulsion for contact lens-intolerant patients. *Eye Contact Lens* 32(2), 109–111 (2006).

58 Willen CM, McGwin G, Liu B, Owsley C, Rosenstiel C. Efficacy of cyclosporine 0.05% ophthalmic emulsion in contact lens wearers with dry eyes. *Eye Contact Lens* 34(1), 43–45 (2008).

59 Rao SN. Reversibility of dry eye deceleration after topical cyclosporine 0.05% withdrawal. *J. Ocul. Pharmacol. Ther.* 27(6), 603–609 (2011).

60 Su MY, Perry HD, Barsam A *et al.* The effect of decreasing the dosage of cyclosporine A 0.05% on dry eye disease after 1 year of twice-daily therapy. *Cornea* 30(10), 1098–1104 (2011).

61 Wilson SE, Perry HD. Long-term resolution of chronic dry eye symptoms and signs after topical cyclosporine treatment. *Ophthalmology* 114(1), 76–79 (2007).

62 Barber LD, Pflugfelder SC, Tauber J, Foulks GN. Phase III safety evaluation of cyclosporine 0.1% ophthalmic emulsion administered twice daily to dry eye disease patients for up to 3 years. *Ophthalmology* 112(10), 1790–1794 (2005).

63 Jain AK, Sukhija J, Dwedi S, Sood A. Effect of topical cyclosporine on tear functions in tear-deficient dry eyes. *Ann. Ophthalmol. (Skokie)* 39(1), 19–25 (2007).

64 Brignole F, Pisella PJ, De Saint Jean M, Goldschild M, Goguel A, Baudouin C. Flow cytometric analysis of inflammatory markers in KCS: 6-month treatment with topical cyclosporin A. *Invest. Ophthalmol. Vis. Sci.* 42(1), 90–95 (2001).

65 Turner K, Pflugfelder SC, Ji Z, Feuer WJ, Stern M, Reis BL. Interleukin-6 levels in the conjunctival epithelium of patients with dry eye disease treated with cyclosporine ophthalmic emulsion. *Cornea* 19(4), 492–496 (2000).

66 Phillips TE, McHugh J, Moore CP. Cyclosporine has a direct effect on the differentiation of a mucin-secreting cell line. *J. Cell. Physiol.* 184(3), 400–408 (2000).

67 Byun YJ, Kim TI, Kwon SM *et al.* Efficacy of combined 0.05% cyclosporine and 1% methylprednisolone treatment for chronic dry eye. *Cornea* 31(5), 509–513 (2012).

68 Mah F, Milner M, Yiu S, Donnenfeld E, Conway TM, Hollander DA. PERSIST: Physician's Evaluation of Restasis((R)) Satisfaction in Second Trial of topical cyclosporine ophthalmic emulsion 0.05% for dry eye: a retrospective review. *Clin. Ophthalmol.* 6, 1971–1976 (2012).

69 Sheppard JD, Scoper SV, Samudre S. Topical loteprednol pretreatment reduces cyclosporine stinging in chronic dry eye disease. *J. Ocul. Pharmacol. Ther.* 27(1), 23–27 (2011).

70 Kara N, Altinkaynak H, Goker Y, Yuksel K, Yildirim Y. Evaluation of corneal morphologic and functional parameters after use of topical cyclosporine-a 0.05% in dry eye. *J. Ocul. Pharmacol. Ther.* 28(6), 593–597 (2012).

71 Perez-Rico C, Germain F, Castro-Rebollo M, Moreno-Salgueiro A, Teus MA. Effect of topical 0.05% cyclosporine A on corneal endothelium in patients with dry eye disease. *Int. J. Ophthalmol.* 6(4), 471–474 (2013).

72 Luschmann C, Herrmann W, Strauss O, Luschmann K, Goepferich A. Ocular delivery systems for poorly soluble drugs: an *in vivo* evaluation. *Int. J. Pharm.* 455(1–2), 331–337 (2013).

73 Rodriguez-Aller M, Kaufmann B, Guillarme D *et al. In vivo* characterisation of a novel water-soluble Cyclosporine A prodrug for the treatment of dry eye disease. *Eur. J. Pharm. Biopharm.* 80(3), 544–552 (2012).

74 Khan W, Aldouby YH, Avramoff A, Domb AJ. Cyclosporin nanosphere formulation for ophthalmic administration. *Int. J. Pharm.* 437(1–2), 275–276 (2012).

75 Di Tommaso C, Valamanesh F, Miller F *et al.* A novel cyclosporin a aqueous formulation for dry eye treatment: *in vitro* and *in vivo* evaluation. *Invest. Ophthalmol. Vis. Sci.* 53(4), 2292–2299 (2012).

76 Peng CC, Chauhan A. Extended cyclosporine delivery by silicone-hydrogel contact lenses. *J. Control Release* 154(3), 267–274 (2011).

77 Kim H, Csaky KG, Gilger BC *et al.* Preclinical evaluation of a novel episcleral cyclosporine implant for ocular graft-versus-host disease. *Invest. Ophthalmol. Vis. Sci.* 46(2), 655–662 (2005).