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Efficacy and safety of 0.1% cyclosporine A cationic emulsion in the treatment of severe dry eye disease: a multicenter randomized trial

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ABSTRACT

Purpose: The SANSIKA study was conducted to assess the treatment effect of 0.1% cyclosporine A cationic emulsion (CsA CE) eye drops on signs and symptoms of patients with severe dry eye disease (DED).
Methods: This was a multicenter, randomized, double-masked, 2-parallel-arm, 6-month phase III study with a 6-month open-label treatment safety follow-up. Patients with severe DED with corneal fluorescein staining (CFS) grade 4 on the modified Oxford scale were randomized to receive once-daily CsA CE (Ikervis®) or its vehicle.
Results: A total of 246 patients were randomized. The proportion of patients achieving ≥2 grades improvement in CFS and a 30% improvement in symptoms (Ocular Surface Disease Index [OSDI]) by month 6 was 28.6% with CsA CE vs 23.1% with vehicle (p = 0.326) (primary endpoint). Assessment of corneal damage showed greater improvement with CsA CE over vehicle in mean adjusted CFS change from baseline to month 6 (-1.764 vs -1.418, p = 0.037). There was a reduction in ocular surface inflammation assessed by human leukocyte antigen DR expression in favor of CsA CE at month 6 (p = 0.021). The mean OSDI change from baseline was -13.6 with CsA CE and -14.1 with vehicle at month 6 (p = 0.858). The main adverse event was instillation site pain (29.2% vs 8.9% in the CsA CE and vehicle groups, respectively), and it was mostly mild.
Conclusions: CsA CE was well-tolerated and effective in improving corneal damage and ocular surface inflammation and confirmed the positive benefit-risk ratio of this new formulation of CsA for the treatment of severe keratitis in DED.

Keywords: Cationic emulsion, Cyclosporine A, Dry eye disease, Randomized trial, Severe keratitis

Introduction

Dry eye is one of the most common ophthalmic diseases, with a prevalence ranging from 5% to 35% (1). The term dry eye disease (DED) describes a multifactorial disease of the lacrimal functional unit that is associated with increased osmolarity of the tear film and inflammation of the ocular surface (2). Tear hyperosmolarity, resulting from reduced aqueous tear flow and/or increased evaporation, drives a vicious cycle

of DED pathology. Tear hyperosmolarity causes apoptosis of cells of the conjunctiva and cornea while also triggering inflammatory cascades that contribute to further cell apoptosis and altered mucin production. This in turn exacerbates tear film instability, leading to more tear hyperosmolarity (3).

Individuals with DED experience eye symptoms including irritation, pain, dryness, foreign body sensation, and visual disturbance (1). The severe form of the disease is characterized by persistent and recurrent symptoms that are known to poorly correlate with the objective clinical findings (4-8). For instance, patients with severe DED may present with decreased symptoms of discomfort, potentially due to downregulation of corneal sensory receptors (7).

Treatment strategies for DED have largely been restricted to instillation of various artificial tear formulations, which typically provide only short-term relief from DED symptoms (9). Despite the fact that topical steroids have shown some promise for improving the signs and symptoms of DED, their

potential benefit is limited by their known iatrogenic ocular side effects (such as intraocular hypertension and cataract) and are thus not recommended for long-term use (10, 11). A therapeutic approach that has received increased attention in recent years is inhibition of the inflammatory responses associated with DED through the use of anti-inflammatory compounds such as cyclosporine A (CsA). Restasis® (Allergan, Inc., Irvine, CA, USA), an anionic oil-in-water emulsion incorporating CsA (0.05%), was approved by the US Food and Drug Administration in 2003 to treat patients with keratoconjunctivitis sicca (12). In Europe, there is currently no medicinal product that has been approved for treatment of DED; however, Santen SAS (Evry, France) has developed a cationic emulsion formulation containing 0.1% (1 mg/mL) CsA (CsA CE) for the topical treatment of severe forms of immune-mediated ocular surface diseases such as DED. This product was registered in 2015 in the European Union for the treatment of severe keratitis in adult patients with DED that has not improved despite treatment with tear substitutes (13). In contrast with Restasis®, CsA CE is a cationic emulsion, the instillation of which results in a long-lasting presence of CsA in the tear film over the entire surface of the eye (14-16). The ocular bioavailability of CsA with the CsA CE formulation is, therefore, higher than with previous CsA formulations (16).

Patients with DED with severe keratitis have an increased risk of infection, vision loss, and impaired quality of life (1). Preclinical and clinical testing of CsA CE has demonstrated a positive benefit-risk ratio in this specific patient population. A phase III confirmatory trial was conducted in patients with severe DED to demonstrate the superiority of CsA CE over its vehicle in simultaneously improving signs and symptoms after 6 months of treatment. In addition, the overall ocular safety of CsA CE was assessed after 6 and 12 months of treatment.

Methods

Participants

Patients included in this study were male and female patients with severe DED aged 18 and older. Eligible patients were those having corneal fluorescein staining (CFS) graded 4 on the modified Oxford scale (from 0 to 5) (17), a Schirmer test score ≥2 mm/5 min and <10 mm/5 min (18), and an Ocular Surface Disease Index (OSDI) score ≥23 (19).

Study design

This study involved a 6-month, multicenter, randomized, double-masked, vehicle-controlled, parallel-group period, followed by a 6-month open-label follow-up period. The study was conducted in 50 centers in 9 European countries (France, Germany, Italy, Spain, Belgium, United Kingdom, Sweden, Austria, and the Czech Republic).

Patients initially underwent a 2-week washout period during which any ongoing ophthalmic treatments were stopped and unpreserved artificial tears (AT) were provided by the sponsor for use as frequently as required throughout the study (saline solution, Larmabak®, Théa, Clermont-Ferrand, France). Patients were randomized to receive one drop once daily at bedtime of CsA CE (Ikervis®) unpreserved single-dose cationic emulsion eyedrops (Santen SAS) or its vehicle for 6 months, according to a 2:1 allocation ratio. During the open-label phase (the last 6 months), all patients received CsA CE. Treatment efficacy and safety were assessed at the end of the 6-month randomized period; safety was also assessed at 12 months following the 6-month open-label period. Intermediate visits also occurred at months 1, 3, and 9.

All enrolled patients provided written informed consent, and the study was conducted in accordance with the principles of Good Clinical Practice and with the ethical principles set out in the Declaration of Helsinki. This study was registered under the following number in the EudraCT database: 2011-000160-97 with the protocol code number NVG10E117 (20).

Efficacy assessments

Efficacy was only determined in the analysis eye, defined as the worst eligible eye.

The same eye (eligible eye) had to fulfill all the applicable aforementioned selection criteria (CFS of 4, Schirmer test score ≥2 mm/5 min and <10 mm/5 min, and OSDI score ≥23). The analysis eye was the eligible eye with the higher lissamine green staining score (17) at baseline. If both eyes had the same lissamine green staining score, the eye with the worse Schirmer test score at baseline was used. If both eyes had the same Schirmer test score, the right eye was used.

To assess the efficacy of CsA CE, both objective (signs) and subjective (symptoms) parameters were examined. Objective assessments included CFS, Schirmer test (without anesthesia), tear break-up time (TBUT) (18), lissamine green conjunctival staining using the Van Bijsterveld scale, tear film osmolarity, and human leukocyte antigen DR (HLA-DR) expression on the conjunctival cell surface by impression cytology (21-23). Subjective assessments included OSDI, visual analogue scale (VAS) of ocular discomfort, use of concomitant AT, investigator's global evaluation of efficacy, and National Eye Institute Visual Function Questionnaire (NEI-VQF-25).

Safety assessments

Safety was assessed by best-corrected distance visual acuity (BCDVA) and intraocular pressure (IOP) in both eyes, blood sampling for CsA levels, vital signs (blood pressure, pulse rate, and respiratory rate), ocular/systemic adverse events (AEs), and slit-lamp examination of both eyes.

Statistical analysis

The primary efficacy endpoint of this study was the combined CFS-OSDI responder rate at month 6. A CFS-OSDI responder was defined as a subject displaying an improvement of ≥2 grades in CFS from baseline and an improvement of ≥30% in OSDI from baseline. This endpoint was analyzed on the full analysis set (FAS) using imputed data with a logistic regression model (factors "treatment" and "pooled country"). The FAS comprised all patients randomized into the study who received any amount of the study drug and were analyzed according to randomized treatment (intention-to-treat principle). Missing data were imputed as follows.

Patients who discontinued treatment before month 6 were considered nonresponders if discontinuation was due to lack of efficacy, lack of tolerance, or change in dry eye therapy. Patients who discontinued before month 1, or who did not discontinue before month 6 but for whom month 1, month 3, and month 6 evaluations were missing, were also considered as nonresponders. If the patient discontinued before month 6 due to a reason other than those specified above, a last observation carried forward (LOCF) procedure was used (carrying forward the month 3 or month 1 evaluation). The LOCF was also used if a patient did not discontinue before month 6 but for whom the evaluation was missing.

Sensitivity analyses were also performed for the primary efficacy endpoint using the primary logistic model on the per-protocol set (PPS), on the FAS using observed data only, and on the FAS considering the actual treatment received and using a Cochran-Mantel-Haenszel (CMH) test controlling for pooled country. The PPS excluded FAS patients with any major protocol deviation.

Further efficacy endpoints were analyzed on the FAS and the PPS. The CFS, OSDI, global VAS, and combined CFS-VAS responder rates and complete corneal clearing rate were analyzed using the logistic model described above. Analysis of CFS, OSDI, global VAS, and lissamine green score change from baseline was performed using a repeated-measures analysis of variance with the following fixed factors: treatment, visit, pooled country, and treatment by visit interaction. Schirmer test, TBUT, NEI-VQF-25, HLA-DR expression, and tear film osmolarity were analyzed using an analysis of covariance model with the fixed factors treatment and pooled country, and the corresponding baseline data as covariate. A supportive analysis was conducted using a CMH test stratified on pooled country. This test was also used to analyze the investigator global evaluation of efficacy.

Sample size calculations were based on the results of a previous phase III study performed in patients with moderate to severe DED (24). The expected CFS-OSDI responder rates at 6 months were 28% and 10% with CsA CE and its vehicle, respectively. Setting the risk α at 5% and the power at 90%, around 225 evaluable patients were needed (150 in the CsA CE group and 75 in the vehicle group) to detect a significant difference between groups. Accounting for nonevaluable patients (approximately 10%), a total of 252 patients were to be recruited.

The safety analysis set was used for reporting safety data; this included all randomized patients for whom there was any evidence they used study medication and for whom any follow-up data were available. Safety analyses were performed using the actual treatment received.

Results

Patient demography

Among the 313 patients who were screened, 261 patients were randomized to receive treatment. A total of 245 patients, 154 in the CsA CE group and 91 in the vehicle group, were included in the full analysis set. The participant flow chart diagram from screening until the completion of the study is summarized in Figure 1. Demographic (age and sex) and baseline disease characteristics (time since DED diagno-
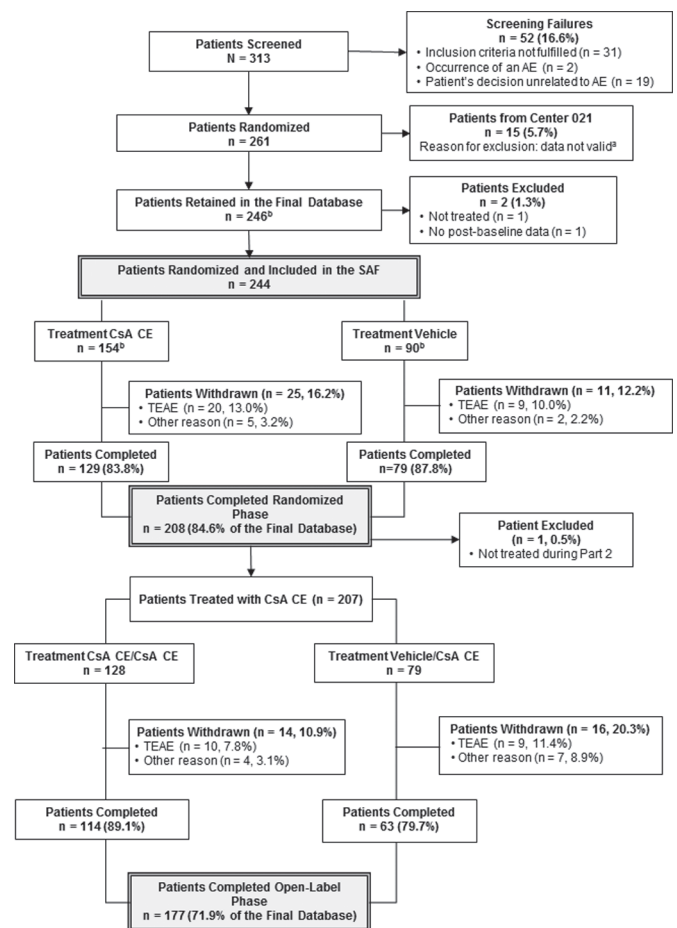

Fig. 1 - Patient flow during the SANSIKA study. [a]Reason for non-retention was major breach to good clinical practice. [b]During the study, 3 patients were dispensed the incorrect treatment: 1 patient randomized to 0.1% cyclosporine A cationic emulsion (CsA CE) received vehicle and 2 patients randomized to vehicle received CsA CE. One patient did not take any study medication and was excluded from the full analysis set (FAS). Thus, the FAS consists of 245 patients: 154 in the CsA CE group and 91 in the vehicle group. AE = adverse event; SAF = safety analysis set; TEAE = treatment-emergent adverse event (defined as an event that started on or after the date of the first study drug dose).

sis, Sjögren syndrome status) were generally well-balanced across the randomized treatment groups (Tab. I). Median use of artificial tears during the screening-baseline period was relatively similar in both treatment groups (9.2 drops/day/eye with CsA CE and 10.2 drops/day/eye with vehicle).

Efficacy results

The results presented below are limited to the double-masked comparative study period.

CFS-OSDI responder rate

This analysis shows no statistically significant difference in the CFS-OSDI responder rate between treatment groups at

TABLE I - Baseline demographic characteristics

	CsA CE (n = 154)	Vehicle (n = 91)	Total (n = 245)
Age, y	n = 154	n = 91	n = 245
Mean (SD)	60.8 (13.5)	62.1 (11.8)	61.3 (12.9)
Median (min, max)	61.7 (22.9, 87.6)	63.5 (32.7, 86.3)	62.5 (22.9, 87.6)
Sex	n = 154	n = 91	n = 245
Female, n (%)	126 (81.8)	83 (91.2)	209 (85.3)
Male, n (%)	28 (18.2)	8 (8.8)	36 (14.7)
Sjögren syndrome	n = 154	n = 91	n = 245
Number (%) of patients	58 (37.7)	34 (37.4)	92 (37.6)
Time since diagnosis, y	n = 153	n = 91	n = 244
Mean (SD)	8.8 (7.1)	9.7 (6.7)	9.1 (7.0)
Median (min, max)	6.2 (0.2, 31.5)	8.7 (0.2, 30.7)	6.8 (0.2, 31.5)

CsA CE = 0.1% cyclosporine A cationic emulsion.
Data represent the full analysis set population.

TABLE II - Responder rates in key efficacy variables after 6 months of randomized treatment with CsA CE or vehicle

	CsA CE (n = 154)	Vehicle (n = 91)	p Value[a]
Primary endpoint			
CFS-OSDI response (improvement ≥2 grades [CFS] and 30% [OSDI])			
Responders	44 (28.6)	21 (23.1)	0.326
Secondary endpoints			
CFS response (improvement ≥2 grades)			
Responders	80 (51.9)	41 (45.1)	0.346
OSDI response (improvement ≥30%)			
Responders	61 (39.6)	36 (39.6)	0.939
VAS response (improvement ≥30%)			
Responders	48 (31.2)	34 (37.4)	0.302
CFS-VAS response (improvement ≥2 grades [CFS] and 30% [VAS])			
Responders	35 (22.7)	19 (20.9)	0.744
Complete corneal clearing (CFS = 0)			
Yes	10 (6.5)	4 (4.4)	0.428

CFS = corneal fluorescein staining graded on a scale from 0 to 5; CsA CE = 0.1% cyclosporine A cationic emulsion; OSDI = Ocular Surface Disease Index questionnaire; VAS = global visual analogue scale assessment of ocular discomfort.
Data represent imputed data on the full analysis set population. Values are n (%).
[a] p Value for treatment effect in the logistic regression model.

6 months (Tab. II). Based on imputed data and according to treatment as randomized, 44 patients (28.6%) with CsA CE and 21 patients (23.1%) with vehicle showed a combined improvement in CFS (by at least 2 grades) and OSDI (by at least 30%) at month 6.

To detect a potential treatment effect on patients showing a marked improvement in CFS over 6 months, a post hoc analysis of the primary efficacy endpoint was undertaken that increased the threshold for improvement of CFS to 3 grades instead of 2 (Fig. 2). When implementing this more stringent criterion, the CFS-OSDI responder rate was statistically significantly higher in the CsA CE group than the vehicle group

(p = 0.016) when considering imputed data. From a clinical point of view, this difference corresponds to a threefold higher likelihood of response with CsA CE treatment than with vehicle treatment after 6 months (odds ratio 2.9, 95% confidence interval [CI] 1.3, 7.7). These results were confirmed when considering observed data.

Corneal fluorescein staining

There was a statistically significant improvement in CFS score over time (main effect of time: p<0.001) in patients receiving either CsA CE or vehicle (Fig. 3). The main effect
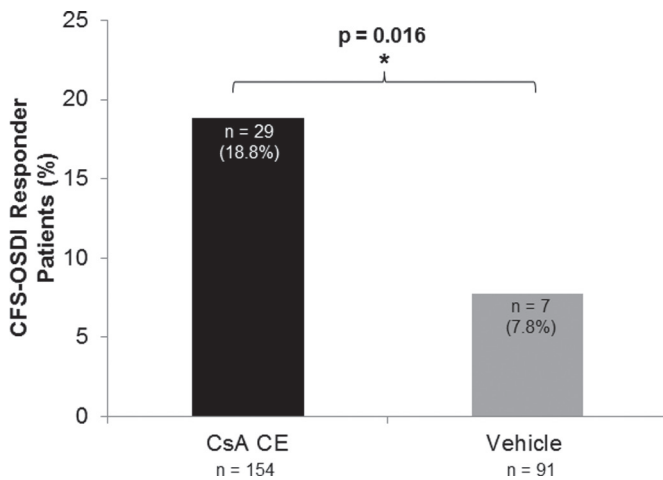
Fig. 2 - Corneal fluorescein staining (CFS)-Ocular Surface Disease Index (OSDI) response rates after 6 months of randomized treatment with 0.1% cyclosporine A cationic emulsion (CsA CE) or vehicle in patients showing a marked improvement in CFS of 3 grades or higher and at least 30% improvement in OSDI. Data represent the imputed data according to the randomized treatment group and the full analysis set population. Comparison between groups was performed using a logistic regression model.
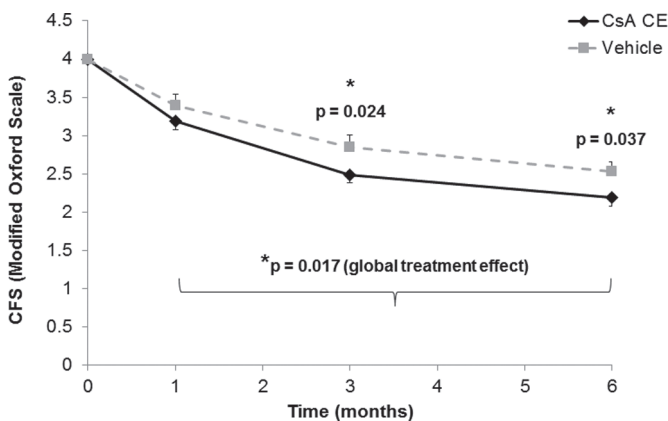

Fig. 3 - Change in corneal fluorescein staining (CFS) over 6 months of randomized treatment with 0.1% cyclosporine A cationic emulsion (CsA CE) or vehicle. Data represent mean CFS values ± standard error of the full analysis set population. Sample size at baseline and months 1, 3, and 6: 154, 149, 140, and 132, respectively, with CsA CE, and 91, 88, 89, and 83, respectively, with vehicle. Comparison between groups was performed using a repeated-measures analysis of variance.

Fig. 4 - Change in human leukocyte antigen DR (HLA-DR) expression over 6 months of randomized treatment with 0.1% cyclosporine A cationic emulsion (CsA CE) or vehicle. Data represent median HLA-DR expression values of the full analysis set population. Comparison between groups was performed using an analysis of covariance model, after logarithmic transformation.

of treatment over a period of 6 months was in favor of CsA CE over vehicle (p = 0.017). The decrease in CFS score from baseline was greater with CsA CE than with vehicle at each time point, reaching statistical significance at month 3 (p = 0.024) and month 6 (p = 0.037). Differences between groups in change from baseline in CFS score were assessed using the adjusted means and observed means. After 6 months of treatment, the adjusted mean change in CFS score from baseline was -1.764 with CsA CE and -1.418 with vehicle, with a difference between groups of 0.35. According to the modified Oxford logarithmic
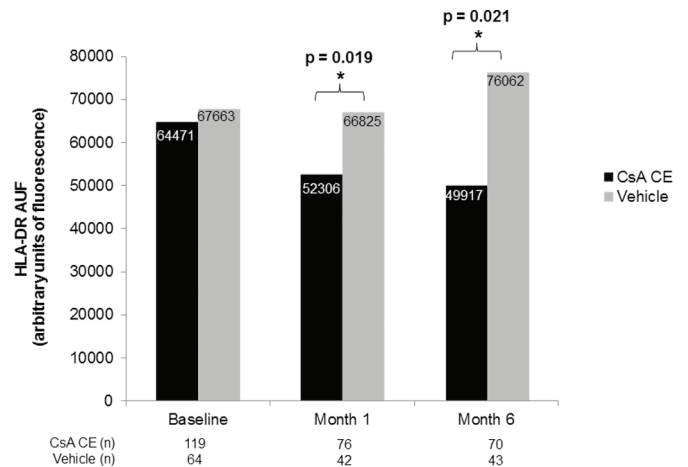
grading scale, the difference of 0.35 represents a ratio of 1.5 in the damaged surface area, corresponding to approximately 50% more punctate dots on the cornea with vehicle compared with CsA CE. Per the observed data at 6 months, the mean change in CFS score from baseline was -1.81 with CsA CE and -1.48 with vehicle, with a difference between groups of 0.33, corresponding to approximately 46% more dots on the cornea with vehicle compared with CsA CE.

The likelihood of CFS improvement by at least 3 grades within 6 months of treatment (post hoc analysis) was approximately 3 times higher with CsA CE than with vehicle (odds ratio 3.3, 95% CI 1.6, 7.0), with 35.6% of the patients reaching grade 1 or less in the CsA CE group compared with 14.5% in the vehicle group (p = 0.001).

Impression cytology

The effect of treatment on inflammation at the conjunctival cell surface was determined by using HLA-DR expression as a biomarker. The decrease in the level of HLA-DR expression from baseline quantified in arbitrary units of fluorescence (AUF) was greater with CsA CE than with vehicle (Fig. 4), with a statistically significant difference at month 1 (p = 0.019) and month 6 (p = 0.021).

Tear film osmolarity

A trend of improvement in both groups was found without statistical difference between groups.

Following experts' recommendations, tear film osmolarity was analyzed post hoc in patients with baseline levels >308 mOsm/L (considering the worst value between both eyes), a threshold known to be indicative of DED (25). A total of 55 patients met this criterion, 34 (22.1%) in the CsA CE group and 21 (23.1%) in the vehicle group, and the mean osmolarity value was similar between these subgroups.

Although there was an improvement in the worst tear film osmolarity over time in both subgroups, the CsA CE subgroup showed a significantly greater change from baseline at month 6 than the vehicle subgroup (p = 0.048), with the mean and median values of worst tear film osmolarity in the CsA CE subgroup lower than 308 mOsm/L, whereas they remained slightly higher than this threshold in the vehicle subgroup (Fig. 5 and supplementary Tab. I, available online at www.eur-j-ophthalmol.com).

Other efficacy analyses

The CFS, OSDI, VAS, and CFS-VAS responder rates and complete corneal clearing rate at month 6 are presented in Table II. The baseline scores and change from baseline at month 6 for OSDI, VAS, and the Schirmer test (without anesthesia) are presented in Table III. Schirmer test results are also presented in Figure 6A. A general trend of improvement in both signs and symptoms was evident in both the CsA CE and vehicle treatment groups. Improvements were greater in the CsA CE group than in the vehicle group for most variables
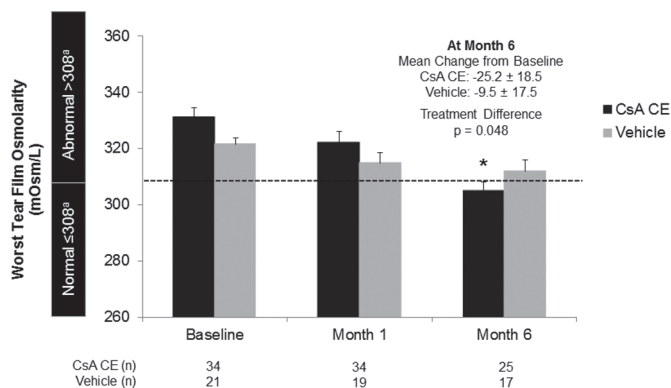

Fig. 5 - Change in worst tear film osmolarity between both eyes in patients with >308 mOsm/L at baseline over 6 months of randomized treatment with 0.1% cyclosporine A cationic emulsion (CsA CE) or vehicle. Data represent mean values ± standard error of a subgroup of the full analysis set population. Means were adjusted for baseline values using an analysis of covariance model with "treatment" and "pooled country" as fixed factors and the baseline data as covariate. [a]Osmolarity ranges taken from Lemp et al (25).

TABLE III - Secondary efficacy assessments: change from baseline after 6 months of randomized treatment with CsA CE or vehicle

	CsA CE (n = 154)	Vehicle (n = 91)	p Value
OSDI score			
Baseline	n = 154	n = 91	
Mean ± SD	61.4 ± 19.4	58.8 ± 18.4	
Median (min, max)	62.5 (25.0, 100.0)	58.3 (25.0, 100.0)	
Change at month 6	n = 131	n = 82	
Mean ± SD	-14.4 ± 21.1	-13.3 ± 18.8	
Adjusted mean (95% CI)[a]	-13.6 (-17.0, -10.0)	-14.1 (-18.6, -9.5)	
Median (min, max)	-14.6 (-79.2, 45.6)	-13.6 (-60.4, 32.5)	0.858
Global VAS assessment (mm)			
Baseline	n = 145	n = 85	
Mean ± SD	55.6 ± 20.6	54.5 ± 18.5	
Median (min, max)	55.1 (5.0, 98.0)	52.5 (16.9, 92.5)	
Change at month 6	n = 120	n = 75	
Mean ± SD	-13.0 ± 22.7	-10.5 ± 21.6	
Adjusted mean (95% CI)[a]	-12.1 (-16.1, -8.2)	-11.2 (-16.3, -6.1)	
Median (min, max)	-11.1 (-59.8, 66.6)	-10.4 (-59.5, 38.5)	0.766
Schirmer test, mm/5 min			
Screening[b]	n = 154	n = 91	
Mean ± SD	3.7 ± 2.0	3.9 ± 2.2	
Median (min, max)	3.0 (2.0, 9.0)	3.0 (2.0, 9.0)	
Change at month 6	n = 141	n = 82	
Mean ± SD	2.2 ± 5.7	1.5 ± 4.3	
Median (min, max)	1.0 (-7.0, 32.0)	1.0 (-5.0, 19.0)	0.604[c]

CI = confidence interval; CsA CE = 0.1% cyclosporine A cationic emulsion; OSDI = Ocular Surface Disease Index questionnaire; VAS = global visual analogue scale assessment of ocular discomfort.
Data represent the full analysis set population. Global VAS assessment was measured on a 0%-100% scale. Because the patient was asked to rate each ocular symptom by placing a vertical mark on a 100-mm horizontal line, data were recorded in mm rather than % (with 1 mm = 1%).
[a] Adjusted means were obtained using a repeated-measures analysis of variance model with the following fixed factors: treatment, visit, pooled country, and treatment by visit interaction.
[b] Values obtained at screening are considered baseline values.
[c] The p value of the nonparametric Cochran-Mantel-Haenszel test was considered instead of the analysis of covariance p value because the distribution of the residuals was not normal (as evaluated by the Shapiro-Wilk test).
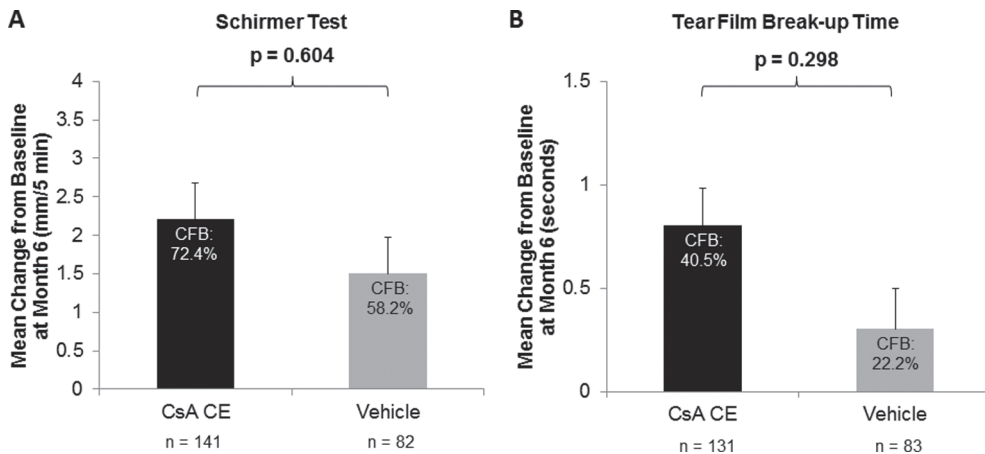
Fig. 6 - Change in Schirmer test (**A**) and tear film break-up time (**B**) over 6 months of randomized treatment with 0.1% cyclosporine A cationic emulsion (CsA CE) or vehicle. Data represent mean values ± standard error of the full analysis set population. For both tests, comparison between groups was performed using nonparametric Cochran-Mantel-Haenszel test instead of analysis of covariance because the distribution of the residuals was not normal (as evaluated by the Shapiro-Wilk test). CFB = change from baseline.

without reaching statistical significance. Schirmer test scores improved by 72.4% and 58.2% with CsA CE and vehicle, respectively (Fig. 6A), but no significant treatment difference was observed. Similarly, the investigator assessment rated the patient's improvement as satisfactory or very satisfactory in a slightly higher proportion of patients assigned to CsA CE (91 patients, 64.1%) than of patients assigned to vehicle (49 patients, 57.0%), but this difference did not reach statistical significance (p = 0.319). A progressive decrease in the use of AT over time was recorded in both treatment groups. Median use of AT during the month 3–month 6 period was 4.4 drops/day/eye with CsA CE (n = 80) and 5.4 drops/day/eye with vehicle (n = 55).

Lissamine green conjunctival staining, TBUT, and NEI-VFQ-25 data (Tab. IV) displayed a similar trend to that observed for most of the secondary efficacy variables. A 40.5% and 22.2% improvement in TBUT was observed with CsA CE and vehicle, respectively, but the difference between treatments was not statistically significant (Fig. 6B). A global trend of improvement in both signs and symptoms was found, with improvements generally greater (without reaching statistical significance) in the CsA CE group than in the vehicle group.

Safety results

A summary of the treatment-emergent AEs (TEAEs) recorded during this study can be found in supplementary Table II (available online at www.eur-j-ophthalmol.com). The TEAEs considered by the investigator to be treatment-related were reported in a higher proportion of patients treated with CsA CE (37.0%) than with vehicle (21.1%), and almost all were ocular. Most TEAEs were of mild or moderate severity, and only one serious TEAE related to treatment was reported (a severely reduced visual acuity that occurred in one patient treated with vehicle). Instillation site pain, the most frequently reported treatment-related ocular TEAE, was reported in a higher proportion of patients treated with CsA CE (29.2%) than with vehicle (8.9%) and was mostly mild (mild: 16.9%, moderate: 8.4%, and severe: 3.9% in the CsA CE group versus 4.4%, 2.2%, and 2.2%, respectively, in the vehicle group). Other than instillation site pain, there were no clear trends for an increased incidence of any ocular or nonocular TEAE

(related or not) with either treatment. The treatment-related ocular TEAEs reported in at least 1.5% of patients in either group were eyelid edema and instillation site erythema (each in 3 patients, 1.9%) in the CsA CE group and photophobia (in 3 patients, 3.3%), eye irritation, and reduced visual acuity (each in 2 patients, 2.2%) in the vehicle group.

Blood sampling revealed that 4 patients treated with CsA CE had measurable CsA levels that were below the upper limit of quantification (≤5 ng/mL), which is considered negligible. Three patients treated with CsA CE had CsA concentrations >5 ng/mL, but these patients were receiving systemic CsA treatment (allowed according to the clinical protocol provided that the treatment remained stable throughout the course of the study). There were no remarkable changes in BCDVA, IOP, or vital signs. No differences were detected between treatments using slit-lamp examination. Safety analyses over 12 months were performed on the 154 patients who received any amount of CsA CE treatment during this time period. Of these patients, 114 (74%) completed the 6-month open-label phase and were thus exposed to CsA CE for 12 months. The safety profile of CsA CE over 12 months was similar to the 6-month treatment profile (supplementary Tab. II) and did not raise any additional safety concerns.

Discussion

The primary objective of this study was to demonstrate the superiority of CsA CE over vehicle in simultaneously improving signs (CFS) and symptoms (OSDI) in patients with severe DED, defined as patients with CFS score of 4 on the modified Oxford scale, Schirmer test score ≥2 mm/5 min and <10 mm/5 min, and OSDI score ≥23, after 6 months of treatment. Although treatment with CsA CE over 6 months was associated with a significant increase in the CFS-OSDI responder rate, a substantial improvement was also observed in patients receiving vehicle treatment, and no statistical difference was detected between groups. Further efficacy analyses revealed a similar trend of improvement in both signs and symptoms of DED in patients treated with either CsA CE or vehicle. For instance, the OSDI improvement in both groups after 6 months was 14 points on average, which can be considered clinically relevant since it is above the minimal clinically important dif-

TABLE IV - Other efficacy assessments: change from baseline after 6 months randomized treatment with CsA CE or vehicle

	CsA CE (n = 154)	Vehicle (n = 91)	p Value
Lissamine green staining[a]			
Baseline	n = 134	n = 79	
Mean ± SD	4.5 ± 2.1	4.6 ± 2.2	
Median (min, max)	4.0 (0.0, 9.0)	5.0 (0.0, 9.0)	
Change from baseline at month 6	n = 114	n = 71	
Mean ± SD	-1.7 ± 2.1	-1.5 ± 2.2	
Adjusted mean (95% CI)[b]	-1.7 (-2.1, -1.267)	-1.4 (-1.9, -0.9)	0.411
Median (min, max)	-2.0 (-6.0, 4.0)	-1.0 (-9.0, 5.0)	
TBUT			
Baseline	n = 154	n = 91	
Mean ± SD	3.3 ± 1.6	3.5 ± 1.7	
Median (min, max)	3.0 (1.0, 7.3)	3.0 (0.0, 8.5)	
Change from baseline at month 6	n = 131	n = 83	
Mean ± SD	0.8 ± 2.1	0.3 ± 1.8	0.298[c]
Median (min, max)	0.5 (-5.0, 8.7)	0.0 (-4.5, 7.8)	
Tear film osmolarity, mOsm/L			
Baseline	n = 57	n = 34	
Mean ± SD	308.1 ± 20.9	305.6 ± 15.5	
Median (min, max)	305.0 (276.0, 366.0)	303.5 (275.0, 339.0)	
Change from baseline at month 6	n = 40	n = 30	
Mean ± SD	-3.3 ± 28.3	-5.8 ± 18.0	0.763[b]
Median (min, max)	-3.0 (-61.0, 118.0)	-7.5 (-38.0, 39.0)	
NEI-VFQ-25 composite score			
Baseline	n = 98	n = 55	
Mean ± SD	71.9 ± 15.74	74.0 ± 13.4	
Median (min, max)	72.9 (11.9, 96.4)	75.8 (43.0, 96.6)	
Change from baseline at month 6	n = 73	n = 46	
Mean ± SD	5.2 ± 8.9	4.8 ± 9.9	
Adjusted mean (95% CI)[d]	4.1 (1.9, 6.2)	4.0 (1.3, 6.6)	0.945
Median (min, max)	4.7 (-15.7, 32.3)	5.0 (-21.2, 24.7)	

CI = confidence interval; CsA CE = 0.1% cyclosporine A cationic emulsion; NEI-VFQ-25 = National Eye Institute Visual Function Questionnaire; TBUT = tear break-up time.
Data represent the full analysis set population.
[a] Data presented do not include data from patients for whom investigators were not able to perform the lissamine green examination correctly.
[b] Adjusted means were obtained using a repeated-measures analysis of variance model with the following fixed factors: treatment, visit, pooled country, and treatment by visit interaction.
[c] The p value of the nonparametric Cochran-Mantel-Haenszel test was considered instead of the analysis of covariance (ANCOVA) p value because the distribution of the residuals was not normal (as evaluated by the Shapiro-Wilk test).
[d] Means were adjusted for baseline values using an ANCOVA model with the fixed factors treatment and pooled country and the baseline data as covariate.

ference described by Miller and colleagues in 2010, which ranges between 7.3 and 13.4 in severe disease (26).

The fact that the superiority of CsA CE over vehicle did not reach statistical significance in the primary endpoint, nor in most of the other efficacy endpoints, may be explained by the ability of the vehicle to improve DED symptoms on its own (5, 27). The vehicle, an unpreserved cationic oil-in-water nanoemulsion, increases the retention time of nanodroplets on the ocular surface by interacting electrostatically with the negatively charged components of the tear film. This property improves drug delivery but also enhances film hydration, lubrication, and stability (14, 16, 28). Additional-ly, efficacy endpoints assessing a concomitant improvement in both signs and symptoms may have been affected by the well-documented weak correlation between signs and symptoms in DED (4, 6, 7). The subjective nature of symptom severity and decreased corneal sensation in severe DED may explain the difficulty in reaching statistical significance in the primary endpoint (6, 29).

Due to the variable and multifactorial nature of the disease, the required length of treatment may vary between individual patients with DED (7, 18), which may also contribute to the difficulty of efficacy evaluation using the combined signs and symptoms endpoint. However, it should be noted

that preclinical evaluations of CsA CE demonstrated maximal corneal and conjunctival CsA penetration at the concentration and posology used in this study (14).

There was a clear trend of greater improvement in patients receiving once-daily CsA CE compared with vehicle in most efficacy assessments. The superiority of CsA CE over vehicle reached statistical significance with regard to improvement in the quality of the corneal surface (CFS score) and the reduction of ocular surface inflammation (HLA-DR expression). The benefit of CsA CE treatment over vehicle in reducing corneal staining (CFS) over time was evident as early as month 3, and the difference between groups at month 6 of 0.35 represents, on average, 50% more corneal staining in the vehicle group compared with the CsA CE group, which is considered clinically relevant. A significant decrease in HLA-DR expression (quantified in AUF) was evident in the CsA CE group even after only 1 month of treatment. Conversely, vehicle treatment had almost no effect on this inflammatory biomarker over 6 months. The effect of CsA CE in this respect is most likely associated with the well-established anti-inflammatory properties of CsA (21, 23, 30-35). In this study, HLA-DR expression was also assessed using percentage of HLA-DR+ cells. The results showed no difference between groups due to the fact that this analysis cannot discriminate a population with a high fluorescence expression from another with a low fluorescence.

Post hoc data analyses provided further support for the superiority of CsA CE over vehicle in the treatment of DED. When considering patients showing a marked improvement in CFS of 3 grades instead of 2 (as was specified in the primary efficacy endpoint), there was a statistically significant difference in the CFS-OSDI responder rate between treatment groups. Additionally, when considering tear film osmolarity in the subgroup of patients who had a baseline value greater than the commonly accepted threshold indicative of DED (25), the CsA CE subgroup showed a significantly greater improvement than the vehicle subgroup.

The CsA CE was generally found to be well-tolerated in most patients, with no systemic findings that could suggest systemic absorption of cyclosporine. Apart from brief instillation site pain, which was more common in patients treated with CsA CE, there were no clear trends for an increased incidence of any ocular TEAE with either treatment. Furthermore, no detrimental effects on visual acuity, IOP, or vital signs were observed.

In conclusion, in the present study, CsA CE (Ikervis®)—a novel formulation of unpreserved single-dose cationic emulsion of cyclosporine 0.1%—was well-tolerated and effective in improving corneal surface damage and ocular surface inflammation. These benefits were achieved with a single daily instillation, which, compared with other DED treatments that require multiple instillations for efficacy, may reduce patient burden and improve quality of life (1). This study confirms the positive benefit-risk ratio of once-daily administration of Ikervis® in the treatment of severe keratitis in patients with severe DED.

Acknowledgment

Disclosures

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ONLINE-ONLY SUPPLEMENTARY MATERIAL

Supplementary Table I. Change in worst tear film osmolarity between both eyes after 6 months randomised treatment with CsA CE or vehicle in patients with baseline values >308 mOsms/L

	CsA CE n=154	Vehicle n=91	p value
Tear film osmolarity (mOsms/L)			
Baseline	n=34	n=21	
Mean±SD	331.0±20.2	321.5±10.5	
Median (min, max)	327.5 (309.0, 385.0)	319.0 (310.0, 346.0)	
Change at Month 6	n=25	n=17	
Mean±SD	−25.2±18.5	−9.5±17.5	
Adjusted mean (95% CI)[a]	−26.7 (−35.0, −18.3)	−16.7(−25.0, −8.4)	p=0.048
Median (min, max)	−24.0 (−72.0, 0.0)	−9.0 (−38.0, 19.0)	

Data represent a subgroup of the FAS population.

CsA CE: 0.1% ciclosporin A cationic emulsion; SD: standard deviation; 95% CI: 95% confidence interval.

[a] Means were adjusted for baseline values using an analysis of covariance (ANCOVA) model with the fixed factors: "treatment" and "pooled country" and the baseline data as covariate.

Supplementary Table II. Summary of adverse events recorded during the study

TEAEs	CsA CE 6 months n=154		Vehicle 6 months n=90		CsA CE 12 months n=154	
	n (%) patients	n events	n (%) patients	n events	n (%) patients	n events
Any TEAE	88 (57.1)	175	42 (46.7)	88	113 (73.4)	275
Any treatment-related TEAE	57 (37.0)	95	19 (21.1)	30	70 (45.5)	128
Any ocular TEAE	66 (42.9)	112	27 (30.0)	44	86 (55.8)	160
Any treatment-related ocular TEAEs	57 (37.0)	90	18 (20.0)	29	70 (45.5)	118
Any TEAE leading to discontinuation[a]	21 (13.6)	34	9 (10.0)	11	31 (20.1)	51
Any ocular TEAE leading to discontinuation	18 (11.7)	29	6 (6.7)	8	27 (17.5)	40
Any severe ocular TEAE	9 (5.8)	16	5 (5.6)	8	11 (7.1)	19
Any SAE[b]	6 (3.9)[b]	6[b]	6 (6.7)	6	14 (9.1)	14
Any treatment-related SAEs	0 (0.0)	0	1 (1.1)	1	0 (0.0)	0
Any ocular SAE	0 (0.0)	0	1 (1.1)	1	0 (0.0)	0
Deaths	0 (0.0)	0	0 (0.0)	0	0 (0.0)	0

Data represent patients who received any amount of treatment over the specified time period.

CsA CE: 0.1% ciclosporin A cationic emulsion; TEAE: treatment-emergent adverse event; SAE: serious adverse event.

If a patient had multiple occurrences of an event, the patient was counted only once in the corresponding patient count.

[a] This category is about TEAEs that led to permanent discontinuation of treatment. All patients who stopped treatment were also discontinued from the study, except one patient who continued the study and completed the 6-month randomised treatment phase.

[b] There was 1 SAE that started during the double-masked period (up to Month 6) but its seriousness (i.e. event requiring hospitalization) was known by the investigators after the double-masked period database lock.