Safety and Efficacy Clinical Trials for SYL1001, a Novel Short Interfering RNA for the Treatment of Dry Eye Disease

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Purpose. To evaluate the efficacy and safety of SYL1001, a short interfering (si) RNA targeting the transient receptor potential cation channel subfamily V member 1 (TRPV1), for the treatment of dry eye disease (DED).

Methods. This study combines a phase I and two phase II clinical trials to test different doses of SYL1001 in a total of 156 healthy subjects and patients with DED. After 10 days of treatment, the primary efficacy endpoints were the effect on (1) the scoring in the Visual Analogue Scale (VAS) and Ocular Surface Disease Index (OSDI) questionnaires, and (2) ocular tolerance evaluated by corneal fluorescein staining and conjunctival hyperemia. Secondary endpoints included the assessment of systemic and local tolerance.

Results. Topical administration of SYL1001 1.125% once daily produced a significant decrease in VAS scores compared with placebo from day 4 until the end of treatment (change from baseline at day 10: -1.73 ± 0.32 vs. -0.91 ± 0.34; $P = 0.013$). For all treatments, OSDI scores were significantly reduced compared to their respective baseline values ($P < 0.01$), although no significant changes were detected between groups. Conjunctival hyperemia (quantified as normal or abnormal) significantly improved after instillation of SYL1001 1.125% compared with placebo (50% vs. 20%; $P < 0.05$). Excellent tolerability was reported, with no differences in the rates of occurrence of adverse events between groups.

Conclusion. These trials achieved their primary endpoints of identifying the most effective dose of SYL1001 (1.125%). SYL1001 showed a large safety margin and may provide novel therapeutic opportunity for the relief of dry eye. (ClinicalTrials.gov numbers, NCT01438281, NCT01776658, and NCT02455999.)

Keywords: siRNA, dry eye disease, ocular pain, visual analogue scale

Dry eye is a common and multifactorial disease characterized by a disruption of the tear film and concurrent ocular surface damage.[1] The leading causes of dry eye disease (DED) include dysfunction of meibomian glands, a decrease in mucin production, chronic blepharitis, refractive surgery, and environmental conditions. Associated symptoms often refers to redness, irritation, itching, and burning eyes, resulting in eye discomfort and pain in most severe cases. Although no uniform diagnostic criteria have yet been established, an estimated 300 million people worldwide are thought to suffer from DED.[2] Very few therapeutic options are currently available to these patients. The first line treatment involves the use of ocular artificial tear substitutes, gels, and ointments, followed by the application of anti-inflammatory agents, and ultimately instillation of serum or umbilical cord serum eye drops.[3] Overall, these agents showed low efficacy in the treatment of DED and the relief of related symptoms, mainly in patients with moderate to severe disorder. Restasis (Allergan, Inc., Irvine, CA, USA) in the United States and Ikervis (Santen, St. Albans, UK) in Europe, are two cyclosporine ophthalmic emulsions approved for the management of chronic dry eye caused by inflammation. Very recently, Xiidra (lifitegrast; Shire, Lexington, MA, USA), an integrin antagonist, has also been approved in the United States for the relief of signs and symptoms of dry eye. However, uncertainty remains about the relative efficacy of these agents compared with placebo. In addition, they have been associated with distinct side effects such as instillation pain and irritation.[4,5]

Table 1. Clinical Trials Design

Phase	Treatment Regimen	No. of Subjects	Number of Instillation (Frequency)	Outcomes
I, Period 1	SYL1001 2.25%, single dose (26.6 μL)	6	1	Primary endpoint: • Ocular surface tolerability (cornea and conjunctiva)
I, Period 2	SYL1001 2.25%, repeated doses (26.6 or 40 μL)	12	7 (one/d)	Secondary endpoints: • Local and systemic tolerability after each study dose
	SYL1001 2.25%, repeated doses (26.6 or 40 μL)	12	7 (one/d)	• Treatment impact on other ocular parameters • Adverse events occurrence • Pharmacokinetics
II (SYL1001_II)	Placebo	20	10 (one/d)	Primary endpoints:
	SYL1001 1.125%, repeated doses	20	10 (one/d)	• Analgesic effect (changes in VAS and OSDI from baseline)
	SYL1001 2.25%, repeated doses	20	10 (one/d)	• Ocular surface tolerance (hyperemia and CFS)
II (SYL1001_III)	Placebo	24	10 (one/d)	Secondary endpoints:
	SYL1001 0.375%, repeated doses	21	10 (one/d)	• Systemic tolerability
	SYL1001 0.75%, repeated doses	21	10 (one/d)	• Treatment impact on other ocular parameters (TBUT) • Adverse events occurrence

Sylentis is developing SYL1001, a novel chemically synthesized 19-base pair small interfering oligonucleotide RNA (siRNA), for the specific inhibition of the transient receptor potential cation channel subfamily V member 1 (TRPV1). The RNA interference (RNAi) technology allows for selective degradation of mRNA and silencing of protein coding genes: this mechanism produces transient silencing lasting for a prolonged period of time, thus providing a valuable tool for the development of new treatment strategies.[6] Indeed, RNAi compounds represent a fast growing class of new pharmaceutical drugs that target disease-related genes. In addition to providing higher specificity than small molecule inhibitors, they can also interfere with the production of proteins not accessible to monoclonal antibodies.[7] Several RNAi compounds have already reached advanced phases of clinical trials in various diseases, including ophthalmic affectations such as glaucoma and macular edema.[8]

A particular complication of DED consists in the appearance of corneal epithelial damage, which in turn stimulates the nerve endings in the cornea to produce eye discomfort.[9] TRPV1, also known as the capsaicin receptor, is a nociceptive transducer involved in the sensing and transmission of pain stimuli.[10] Interestingly, it has also been shown to participate in the modulation of inflammatory response.[11,12] TRPV1 is found expressed in various eye tissues including the corneal epithelium and the basal layer of the conjunctiva, hence representing an important mediator of ocular pain signal.[12] The current study describes the results obtained in phase I and II clinical studies, which aim to determine the safety and efficacy of SYL1001, a topically administered siRNA targeting TRPV1 expression on the ocular surface.

Materials and Methods

The study was conducted in accordance with the Declaration of Helsinki and with International Conference in Harmonization Guidelines on Good Clinical Practices CMP/ICH/135/95. Subjects signed a written consent form stating that they understood and agreed to participate in the clinical study. The clinical trials were registered on www.clinicaltrials.gov (Phase 1: NCT01438281; Phase II SYL1001_II: NCT01776658; and SYL1001_III: NCT02455999).

SYL1001 Synthesis and Quantification

SYL1001 is a synthetic, 19-base pair double-stranded RNA oligonucleotide duplex formulated in PBS (pH 7.2). SYL1001 against human TRPV1 mRNA consisted in the following sequence: sense 5′-AAGCGCAUCUUCUACUUCA-3′ and antisense 3′-UGAAGUAGAAGAUGCGCUU-5′. siRNAs were synthesized by Biospring (Frankfurt am Main, Germany). For clinical trials, solutions were aliquoted into sterile single-dose vials at GP Pharm (Barcelona, Spain), as preservative-free solution. The vehicle formulation was used as placebo. Quantification of SYL1001 in plasma samples was performed using the ultra-performance liquid chromatography–tandem mass spectrometry (UPLC MS/MS); this method was validated at Harlan Laboratories (Barcelona, Spain). The concentration interval of the bioanalytical procedure was 10 ng/mL (lower limit of quantification, LLOQ) to 500 ng/mL (upper limit of quantification, ULOQ).[13]

Clinical Trials Study Design

This report combines a phase I and two phase II (named SYL1001_II and III) clinical trials. The phase I study was a single-center, randomized, parallel, intrinsically-controlled, open-label trial to assess the safety, tolerability, and bioavailability of ocular SYL1001 at 2.25% after topical administration in healthy volunteers (Table 1). The phase II clinical trials were multicenter, randomized, placebo-controlled and double masked studies to assess the safety and efficacy of SYL1001 in patients with DED. The SYL1001_II study was conducted at six sites in Spain; SYL1001_III trial was performed in Spain and Estonia at four and two sites, respectively. Both were dose-finding studies, testing SYL1001 at 1.125% or 2.25% in the trial SYL1001_II, and 0.375% or 0.75% in the trial SYL1001_III, against placebo (see Schedule in Table 1).

Subjects

The phase I clinical trial included thirty healthy volunteers of both sexes aged between 18 to 38 years who had IOP values below 21 mm Hg, logMAR visual acuity of 0.10 or better, and presented normal values in fluorescein clearance test (FCT) and in ocular fundoscopy. In all cases, the drug was instilled to one randomly chosen eye. Both the randomized treated eye prior to administration and the untreated eye served as a control for ocular tolerance and safety evaluations. Both eyes were monitored in a masked fashion. The first patient first visit (FPFV) was July 28, 2011 and the last patient last visit (LPLV) November 28 2011.

In the combined safety and efficacy trials, a total of 126 patients were randomly allocated to the different treatment

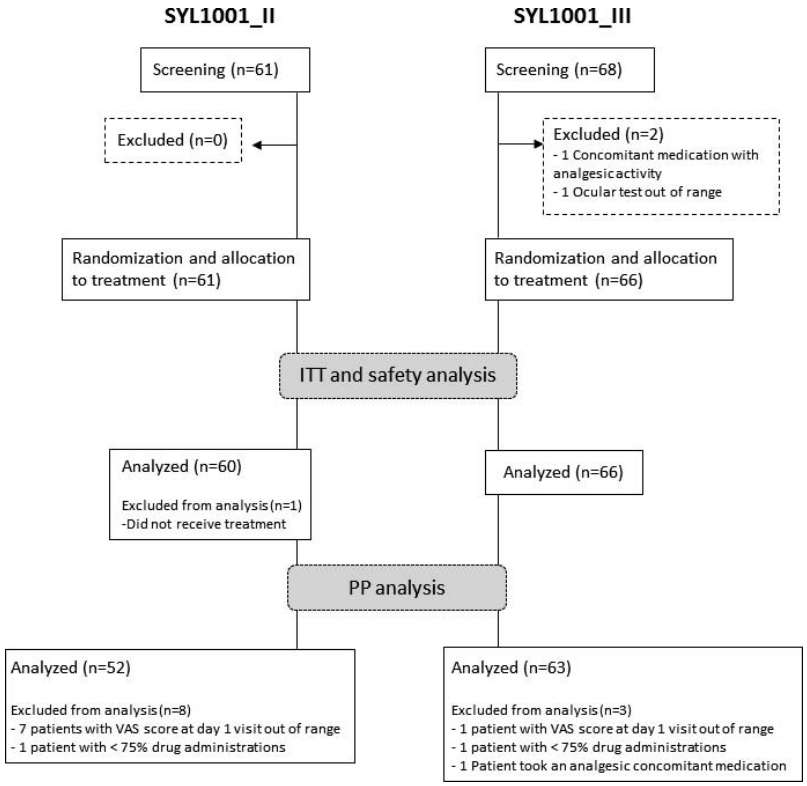

Figure 1. Participant flow chart diagram. The total number of patients screened in the combined phase II trials (SYL1001_II and SYL1001_III) was 129, and allocation to treatment was 126. One patient did not receive medication and was excluded for the safety analysis. The Intention to Treat and safety analysis comprises all subjects who were randomized to any of the study treatments and received at least one dose of study medication. Per protocol set is defined as all subjects who did not experience a major protocol violation.

groups as depicted in Table 1. The sample size was based on practical considerations with the aim of detecting a signal. At a two-sided significance level (α) of 5% and power of 90%, 20 patients per arm were considered sufficient to detect significant differences, if any, between the doses. Sixty patients were randomized in the SYL1001_II study and 66 patients in the SYL1001_III trial (Fig. 1; Table 1). Inclusion criteria were patients of both sexes aged over 18 years with mild to moderate dry eye lasting for more than 3 months. Symptoms scores were Ocular Surface Disease Index (OSDI; Allergan, Inc.) between 13 and 70, and Visual Analog Scale (VAS) between 2 and 7. Ocular parameters 15 days prior to inclusion were: (1) Oxford scale $\geq$1, 2) tear break up time (TBUT) $<$10 seconds, and (3) Schirmer's test with anesthesia less than 10 mm/5 minutes. Relevant exclusion criteria included but were not limited to: previous refractory surgery, use of contact lenses during the treatment and the preceding 15 days, change in the pre-established administration schedule of artificial tears, concomitant use of other medications with analgesic activity, and initiation of treatment with cyclosporine. Subjects who fulfilled one of the following conditions were removed from the study: withdrawal of the informed consent, major protocol violation or intolerable adverse event. In all studies, comprehensive physical and ocular tests were performed in order to insure that volunteers met all inclusion/exclusion criteria. FPFV-LPLV for SYL1001_II was February 18, 2013 to April 30, 2015. FPFV-LPLV for SYL1001_III was June 29, 2015 to December 10, 2015.

Treatment Schedule

In accordance with the Guidelines on Strategies to Identify and Mitigate Risks for First in Human Clinical Trials with Investigational Medicinal Products (EMEA/CHMP/SWG/28367/07), the phase I study was divided into two distinct periods, as previously described.[13] Briefly, during the first period, six patients were first treated with a single dose of SYL1001 and observed for 72 hours (Table 1). During the second period, multiple doses of SYL1001 were administered in one randomized eye of each of 12 other patients, once a day for 7 days. In the Phase II clinical trials, the screening visit took place within a period of 15 days prior to the first day of administration. Treatment was allocated following a pre-established randomization list using a random design by blocks. Randomized patients were coded with a 5-digit identifier, and packaging was identical regardless of the treatment arm. Masking codes were sent to the reporting statistician by the packaging company after database lock following conclusion of treatment. Clinical study information for randomized trials is summarized in Supplementary Table S1.

The treatment was instilled in both eyes at the Investigational Centers during the morning for 10 consecutive days with either 40 μL of placebo or SYL1001 at 0.375%, 0.75%, 1.125%, and 2.25 % (Table 1). At least 1 hour after the last drug instillation on Day 10, all safety and ocular parameters were reassessed. A follow-up visit for the assessment of AEs and symptoms progression was conducted personally or by phone between days 14 and 20.

Assessment of Tolerability and Outcome Measures

The primary and secondary safety endpoints of the phase I trial are presented in Table 1. Ophthalmic evaluation and comprehensive clinical evaluations were performed during the screening period and upon final physical examination in each

Table 2. Demographic and Other Baseline Characteristics of Patients in the Safety Phase I Trial

	Period 1	Period 2	
Parameters	26.6 µL SYL1001 2.25%	26.6 µL SYL1001 2.25%	40 µL SYL1001 2.25%
Age, y	26.7 ± 6.4	21.5 ± 3.4	22.6 ± 4.5
Weight, kg	60.6 ± 7.6	67.4 ± 9.8	65.4 ± 9.6
Height, m	1.6 ± 0.1	1.7 ± 0.1	1.7 ± 0.1
BMI, kg/m^2	22.6 ± 2.0	23.0 ± 2.3	23.0 ± 2.3
Sex, % male/ female	17/83	58/42	33/67

Data are presented as mean ± SD.

of the study intervals, as described previously.[13] The primary objectives of the Phase II studies were to evaluate the effect of SYL1001 on VAS and OSDI scores, and to assess the ocular tolerance by recording the frequency of conjunctival hyperaemia occurrence and change in Corneal fluorescein staining (CFS). Ophthalmic examination (including Schirmer's test and TBUT) and clinical test were performed for safety evaluation as secondary objectives. The OSDI questionnaire was first used to evaluate the severity of the disease. The intensity of ocular pain was assessed using a VAS with a scale of 0 (no ocular discomfort) to 10 (maximum eye pain). At the screening visit, these two questionnaires were filled before performing any ophthalmic evaluation. For TBUT, a drop of 2% fluorescein solution was instilled and the interval between the last blink and the first evidence of tear film disruption was recorded once. For safety evaluation, conjunctival hyperemia was ranked as normal or abnormal, blepharitis in the anterior chamber as present or absent, and tear meniscus as normal or thin by a masked investigator. Corneal fluorescein staining was graded using the Oxford scale (0–4). The Schirmer's test was assessed with anesthesia during 5 minutes. All evaluations were made by a masked investigator. For CFS and hyperemia, the percentage of patients that showed improvement, maintenance, or worsening in respect to total group population was calculated. During the treatment schedule, VAS was performed by the patient at each visit before drug administration. Ophthalmic and clinical evaluation at day 10 was performed in the same order and at similar time of the day as the screening visit.

Statistical Analysis

For clinical trial data, normal limits were assessed using Shapiro-Wilk test. All analyses of the continuous efficacy variables within each group (before and after treatment) were performed by 1-sample *t*-Test (or Wilcoxon signed-rank test in case of not normally distributed data). Differences among groups were evaluated by repeated measurements ANOVA, with the treatment group adjusting for others factors (eye and time). Treatment groups were tested at the two-sided 5% significance level. Corneal fluorescein staining and hyperemia were categorized as improvement, worsening, and maintenance from initial to day 10 visit and summarized by treatment group. Categorical safety endpoints were analyzed using the χ^2 test (or log-likelihood ratio test) and continuous variables using an ANOVA analysis (or Kruskal-Wallis test as appropriate). The right eye was selected for the analyses in the phase II studies. For VAS evaluation, results obtained from day 1 to day 10 prior to administration (corresponding to 0–9 instillations) were used. The SAS software Version 9.3 (SAS Institute, Cary, NC, USA) was used for all statistical analyses.

Results

Clinical Safety and Tolerability

Thirty subjects were included in the Phase I safety study; demographic characteristics are presented in Table 2. The comprehensive ocular analysis did not reveal any clinically significant changes in the tested parameters, at any of the doses tested and either scheduled periods (Supplementary Table S2). The differences in IOP and in pupil diameter observed in one treated and one untreated eye, respectively, were assessed as not related to SYL1001. Sixteen mild AEs were reported in 13 healthy volunteers throughout the study, all of which resolved during the study period. All AEs were considered by the investigator as not related to the investigational medication or as unlikely related in the case of foreign body sensation. Importantly, no significant differences in the occurrence or frequency of AEs were observed between treated and untreated eyes ($P = 0.317$). Finally, when comparing the screening with the final periods, no clinically significant changes were observed in the results of physical examination, laboratory parameters, vital signs, and electrocardiogram (ECG); all parameters remained within standard limits (data not shown). Pharmacokinetic analysis showed that SYL1001 could not be detected in any of the collected plasma samples at all distinct time-points following ocular instillation (LLOQ <10 ng/mL). Of interest, previous stability analysis demonstrated that the half-life of SYL1001 is much longer in human aqueous humour than in serum (up to 40 times; data not shown). This suggests that the compound is stable enough to exert its effect in the eye but is rapidly degraded when reaching the systemic circulation.

Similar safety findings were made in the combined tolerability and efficacy trials; demographic data and flowchart of patient inclusion are presented in Table 3 and Figure 1, respectively. In the group of 60 patients that were treated in the SYL1001_II study, eight possibly or probably related AEs were reported in a total of five patients (Table 4). In the SYL1001_III study, 66 patients received a treatment and a total of seven possibly or probably medication-related AEs were recorded (Table 4). One patient receiving the SYL1001 0.75% formulation discontinued the treatment due to abnormal eye sensation and headache, both of which were of moderate severity with a possible link to the medication. No serious AEs were reported in all of the clinical trials. Most reported AEs were of mild intensity and resolved without causing deviations from the study protocol. Finally, no significant differences in AE frequency were observed between treatment groups. Overall, these results suggest that SYL1001, at each of the tested doses, displays good local and systemic tolerability.

Efficacy

Baseline data recorded at the screening visit during the phase II clinical trials are presented in Table 3; no statistically significant differences were found between all groups at this time-point. With regard to the OSDI test, a significant decrease of approximately 30% in total score from initial visit to day 10 was detected for all groups ($P < 0.05$, Fig. 2A, left panel). However, no differences were observed between treatments. Ocular Surface Disease Index scores computed during the last visit are presented in Table 5. The differences in mean change between the initial and last visits were similar in all groups (Fig. 2A, right panel). In order to obtain a more detailed comparison, questions from the OSDI questionnaire were divided into two categories (related to vision or ocular discomfort), however no significant differences were detected between groups (data not shown). These data indicate that

Table 3. Demographic and Other Baseline Characteristics of Patients at Screening Visit in Phase II Trials

	SYL1001_II			SYL1001_III		
Parameters	**Placebo, $n = 20$**	**1.125%, $n = 20$**	**2.25%, $n = 20$**	**Placebo, $n = 24$**	**0.375%, $n = 21$**	**0.75%, $n = 21$**
Age, y	43.1 ± 16.2	48.9 ± 16.5	56.7 ± 16.1	47.8 ± 12.9	45.2 ± 14.9	46.1 ± 13.9
Sex, M/F	25/75	15/85	10/90	8/92	14/86	19/81
Visual acuity, BCVA	0.95 ± 0.2	0.97 ± 0.1	0.98 ± 0.1	0.98 ± 0.1	1.0 ± 0.0	0.98 ± 0.1
IOP, mm Hg	14.1 ± 2.5	15.4 ± 2.5	13.6 ± 3.2	14.9 ± 2.4	15.3 ± 2.9	14.3 ± 2.6
Blepharitis, % patient; P/A	55/45	55/45	65/35	58/42	28/62	48/52
Tear meniscus, % patient; N/T	50/50	45/55	35/65	33/67	29/71	48/52
Hyperemia, % patient; N/A	55/45	35/65	75/25	67/33	40/60	48/52
OSDI	40.7 ± 12.7	37.5 ± 14.4	45.0 ± 11.4	47.3 ± 14.7	42.8 ± 16.6	45.2 ± 15.4
TBUT, s	5.4 ± 2.1	4.25 ± 2.2	4.18 ± 1.7	5.3 ± 1.9	5.3 ± 1.6	4.8 ± 2.4
CFS, Oxford scale; %						
I	70	60	65	69	69	76
II	25	35	30	25	24	24
III	5	2	5	6	7	0
Schirmer's test, mm	5.0 ± 3.1	4.7 ± 2.9	5.3 ± 2.7	4.8 ± 2.8	5.7 ± 2.4	6.1 ± 2.7

Results are displayed as mean ± SD. M/F, male/female; BCVA, best corrected visual acuity; P/A, present/absent (% of patients); N/T, normal/thin (% of patients); N/A, normal/abnormal (% of patients); Oxford scale I to IV (% of patients with improvement).

both SYL1001 and placebo are associated with a decrease in OSDI score.

The degree of ocular pain was assessed before and after treatment with SYL1001 or placebo. Visual Analogue Scale scores at day 10 were significantly lower than at day 1 in all groups ($P < 0.05$). Interestingly, the comparison of VAS scores demonstrated a decrease in ocular pain after instillation of SYL1001 1.125% (SYL1001_II trial) compared with placebo, while the other doses did not produce a statistical significant effect (Fig. 2B, left panels). Significant differences were found from day 4 on, and VAS values remained fairly constant over the remaining course of treatment ($P < 0.05\%$). Mean change in VAS scores between day 10 and day 1 also indicate a significant improvement in ocular pain after treatment with the SYL1001 1.125% dose ($P = 0.013$, Fig. 2B, right panels).

Interestingly, conjunctival hyperemia showed improvement from abnormal to normal grade in 50% of the eyes 10 days after instillation with SYL1001 1.125%, the difference was statistically significant compared with that of placebo ($P < 0.05$, Table 5). As for corneal staining, 70% of patients showed improvement of at least 1° on the Oxford scale after treatment with SYL1001 1.125% compared with 50% for placebo; however, this difference did not reach the level of statistical significance ($P = 0.07$, Table 5). When an improvement of at least 2° was considered, improvement was reached by 40% of the patients treated with SYL1001 1.125% compared with 15% for placebo and 0% for SYL1001 2.25%; while the difference between SYL1001 1.125% and SYL1001 2.25% was statistically significant ($P = 0.004$), the difference with placebo was not, possibly due to small sample size (Table 5). Surprisingly, administration of SYL1001 at 0.75% and 1.125% significantly increased the change in TBUT at day 10 compared with baseline ($P < 0.05$, Table 5). However, no significant differences in mean change were found between groups. Summary data for conjunctival hyperemia, CFS, and TBUT are presented in Figure 3. Finally, blepharitis, Schirmer's test, tear meniscus, IOP, and best corrected visual acuity (BCVA) did not show any clinically significant changes (data not shown).

Discussion

The current study demonstrates that topical SYL1001, a new siRNA compound targeting TRPV1, can reduce ocular pain and conjunctival hyperemia in patients with DED. Moreover, SYL1001 was found to have an excellent safety and tolerability profile at all tested doses (0.375%, 0.75%, 1.125%, and 2.25%).

In the phase I clinical trial, no treatment-related changes were detected in all of the ocular safety parameters. Moreover,

Table 4. Possible or Probable Related Adverse Events Observed in Phase II Trials

	SYL1001_II			SYL1001_III		
Adverse Events	**Placebo ($n = 20$)**	**1.125% ($n = 20$)**	**2.25% ($n = 20$)**	**Placebo ($n = 24$)**	**0.375% (n =21)**	**0.75% ($n = 21$)**
At least one	6/3 (15%)	2/2 (10%)	0/0 (0%)	5/2 (8%)	0/0 (0%)	2/1 (5%)
Local AEs						
Ocular pruritus		2/2 (10%)				
Eye pain				2/2 (8%)		
Abnormal sensation in eye						1/1 (5%)
Ocular discomfort				1/1 (4%)		
Ocular hyperemia				1/1 (4%)		
Systemic AEs						
Nausea	2/2 (10%)					
Dizziness	3/3 (15%)					
Headache	1/1 (5%)			1/1 (4%)		1/1 (5%)

Results are presented as: number of events by category/number of patients in each category (percentage of subjects).

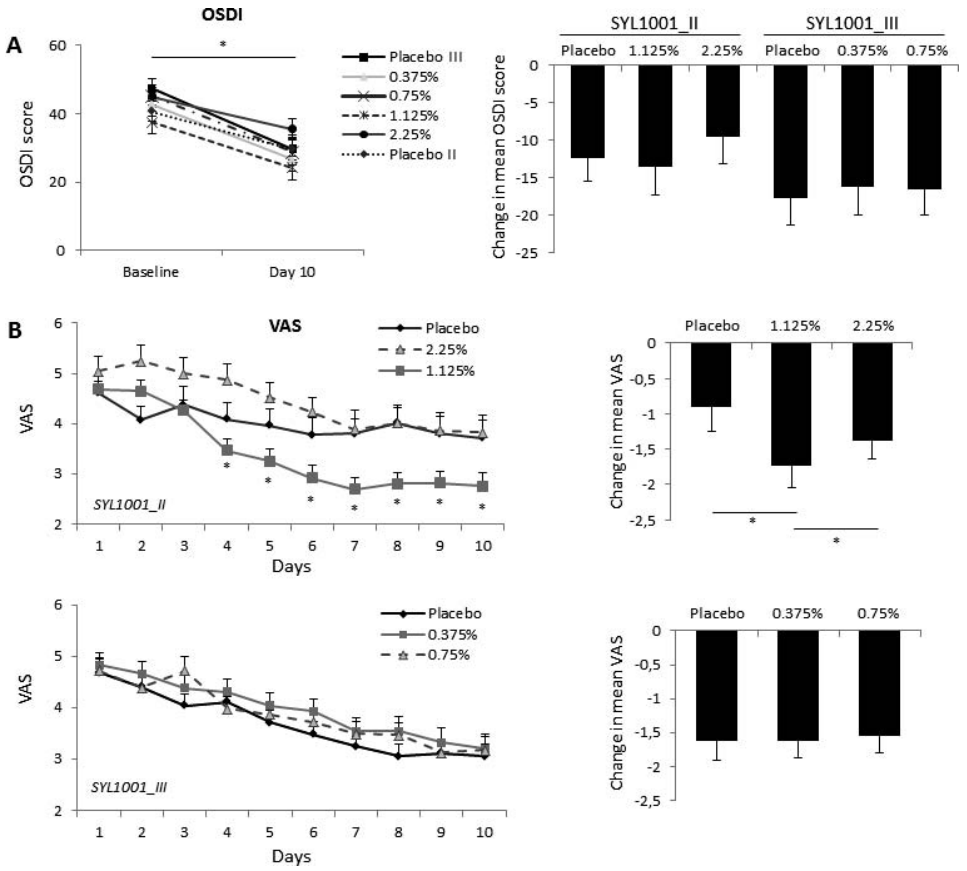

Figure 2. Effect of SYL1001 on OSDI and VAS primary outcomes. (**A**) Ocular Surface Disease Index scores measured before and after treatment for each treatment group (*left*) (*$P < 0.05$ compared with baseline), and mean change in OSDI scores before and after treatment (*right*) for both phase II clinical trials. (**B**) The VAS measured at each treatment day (*left*) and mean change in VAS between the first and last visits. *$P < 0.05$ compared with placebo. Data are mean ± SEM.

serum levels of SYL1001 in treated volunteers were always below detection limit, consistent with the rapid degradation of siRNAs by serum ribonucleases; this represents a major advantage for a locally administered compound as it avoids any possible systemic toxicity. In the phase II trials, all reported AEs were of mild intensity, and no differences in AEs occurrence were observed between treatment and placebo groups. These safety results are in line well with those previously obtained by our group in nonclinical safety studies, as well as in trials using siRNA in ophthalmic solutions.[13] Taken together, these observations indicate excellent safety and local tolerability for SYL1001.

Table 5. Ocular Parameters Recorded at the End of the Treatment in Phase II Trials

	SYL1001_II			SYL1001_III		
Parameters	**Placebo, $n = 20$**	**1.125%, $n = 20$**	**2.25%, $n = 20$**	**Placebo, $n = 24$**	**0.375%, $n = 21$**	**0.75%, $n = 21$**
OSDI (final)	28.4 ± 17.4	24.1 ± 14.1	35.5 ± 16.0	29.7 ± 16.6	26.6 ± 16.5	28.7 ± 17.6
Hyperemia, %						
Improvement	20%	50%*	10%	4%	9%	24%
Maintenance	70%	40%	85%	92%	86%	67%
Worsening	10%	10%	5%	4%	5%	9%
CFS						
>1° Improvement	50%	70%	55%	50%	57%	43%
>2° Improvement	15%	40%†	0%	4%	0%	10%
TBUT						
Final	5.8 ± 3.4	6.4 ± 4.4	4.5 ± 1.8	5.4 ± 2.7	5.3 ± 1.9	6.5 ± 4.2
Mean change	0.43 ± 3.5	2.15 ± 4.4‡	0.3 ± 2.0	0.08 ± 2.1	0.0 ± 2.4	1.67 ± 3.6‡

Data are mean ± SD.
* $P < 0.05$ as compared with placebo.
† $P < 0.01$ as compared with SYL1001 2.25%.
‡ $P < 0.05$ day 10 vs. baseline.

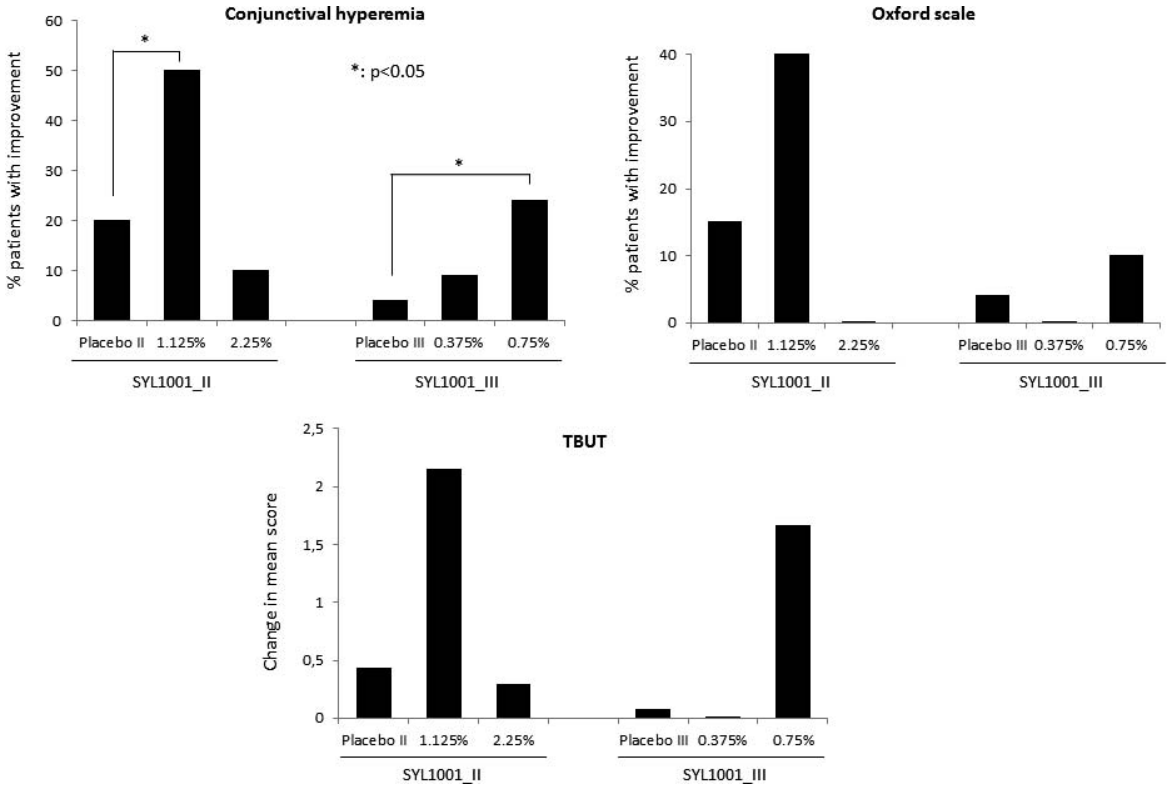

FIGURE 3. Effect of SYL1001 on local primary and secondary outcomes. Percentage (%) of patients showing (**A**) improvement in conjunctival hyperemia, (**B**) improvement by 2° in Oxford score. (**C**) Change in mean TBUT in the two phase II trials. Data are mean scores. $^*P < 0.05$ compared with placebo.

The phase II trials also met their primary efficacy endpoints. Hence, these studies demonstrate that SYL1001 1.125% significantly reduced ocular pain scores, as measured by VAS, from day 4 to the end of treatment compared with placebo. The delay in reaching a statistically significant reduction probably results from the long half-life of the receptor. Of note, the fact that intermediate doses of SYL1001 produced greater effect than higher doses is consistent with the mechanism of action of siRNA. The adequate dose to be administered remains a most challenging problem in RNAi technology: the siRNA must efficiently enter the target cells for further processing by the RNAi silencing complex, processes that may lead to saturation.[14] It follows then, that the dose of siRNA ought to be high enough to produce gene knockdown, but the concentration of the compound at the target site should not compromise endocytosis and processing by the RISC complex. In support of this evidence, preclinical biodistribution analysis performed by our group revealed that maximal amounts of SYL1001 are detected in the cornea and conjunctiva of rabbit eyes after administration of SYL1001 at 1.125%, whereas higher concentrations did not result in greater penetration (data not shown). Finally, other clinical studies have also found great variability in the response to different doses of specific siRNA.[15]

An improvement in OSDI scores was observed after treatment with SYL1001; however, its magnitude was similar to that observed with placebo. Comparable findings have been reported in other trials that failed to find improvement in OSDI score after treatment compared with placebo.[16,17] Through the selection of subjective endpoints related to eye dryness and discomfort, recent studies achieved more clinically significant results.[18,19] Consequently, a growing number of reports now focus on these types of questionnaires, such as the new Ora Calibra Ocular Discomfort scale, which are generally more consistent with the symptoms reported by patients with DED.[20,21]

In the present study, a placebo effect was detected for both the VAS and OSDI parameters, which is consistent with previous reports that also observed an effect of placebo in the relief of eye dryness and discomfort, and even on CFS.[18,22] Herein, the two phase II trials included patients with mild to moderate DED; of interest, patients suffering from mild dry eye usually find relief in the use of artificial tears, and such impression might have also been achieved through the use of the placebo. In order to substantiate this hypothesis, the data from both groups (treatment and placebo) were dichotomized based on low (≤ 4) or high (>4) initial VAS; the subgroup analysis showed that in patients with high VAS, the decrease produced by SYL1001 1.125% was more pronounced than that produced by the placebo ($P = 0.0027$, data not shown). This observation suggests that future studies should be conducted in more advanced cases of DED. Such patients have already been selected in different studies, such as the ones evaluating the efficacy of cyclosporine (OSDI scores ≥ 23), Lifitegrast (VAS $\geq 40/100$), MIM-D3 (TBUT ≤ 5 seconds), and Rebamipide (Schirmer ≤ 5).[5,18–20]

In addition to pain stimuli, SYL1001 may prevent the activation of TRPV1 caused by tear hyperosmolarity. Such activation has been shown to induce the release of proinflammatory cytokines such as IL-6, IL-8, and IL-1β.[11] These inflammatory mediators cause epithelial damage, loss of goblet cells, and deregulation of mucin expression that together lead to tear film instability, which might explain the extended TBUT found after treatment with SYL1001. Our study showed an improvement in conjunctival hyperemia and in CFS after exposure to SYL1001 1.125%, suggesting a link with the underlying signalling pathways.[23] Due to the potential

inhibitory effect of TRPV1 on fibrosis,[24] we have performed preclinical studies in a rabbit model of corneal wound healing and showed that such process is not affected by SYL1001. Moreover, the transparency of the cornea was found to be increased after treatment with SYL1001 (data not shown).

Taken together, these data suggest an advantage for the use of SYL1001 over cyclosporine eye drops (Ikervis), which was only proved to decrease the expression of one inflammatory marker, with minimal improvement in CFS, and nonsignificant trends for osmolarity and eye discomfort (OSDI and VAS).[5] Another advantage for SYL1001 is its excellent safety profile, with a low proportion of patients with medication-related AEs (4% in each arm) compared with Ikervis (37%). The most frequently reported AE of cyclosporine was pain at the instillation site, which is liable to reduce compliance to treatment. Because the SYL1001 trials were dose finding studies, some of the limitations include a short treatment period and small sample size. However, future trials should allow a more robust demonstration of the efficacy of this siRNA.

Overall, the intrinsic properties of TRPV1 confirm its selection as a suitable candidate for targeting pain-related diseases. In this project, we have developed a new siRNA compound targeting TRPV1, formulated in a sterile ophthalmic solution, that can reduce ocular pain scores assessed by VAS, as well as conjunctival hyperemia associated to dry eye.

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References

1. The definition and classification of dry eye disease: report of the Definition and Classification Subcommittee of the International Dry Eye WorkShop (2007). *Ocul Surf*. 2007;5:75–92.
2. The epidemiology of dry eye disease: report of the Epidemiology Subcommittee of the International Dry Eye WorkShop (2007). *Ocul Surf*. 2007;5:93–107.
3. Management and therapy of dry eye disease: report of the Management and Therapy Subcommittee of the International Dry Eye WorkShop (2007). *Ocul Surf*. 2007;5:163–78.
4. Schultz C. Safety and efficacy of cyclosporine in the treatment of chronic dry eye. *Ophthalmol Eye Dis*. 2014;6:37–42.
5. Leonardi A, Van Setten G, Amrane M, et al. Efficacy and safety of 0.1% cyclosporine A cationic emulsion in the treatment of severe dry eye disease: a multicenter randomized trial. *Eur J Ophthalmol*. 2016;26:287–296.
6. Lu PY, Xie F, Woodle MC. In vivo application of RNA interference: from functional genomics to therapeutics. *Adv Genet*. 2005;54:117–142.
7. Fujita Y, Takeshita F, Kuwano K, Ochiya T. RNAi therapeutic platforms for lung diseases. *Pharmaceuticals (Basel)*. 2013;6:223–250.
8. Guzman-Aranguez A, Loma P, Pintor J. Small-interfering RNAs (siRNAs) as a promising tool for ocular therapy. *Br J Pharmacol*. 2013;170:730–747.
9. Rosenthal P, Borsook D. The corneal pain system. Part I: the missing piece of the dry eye puzzle. *Ocul Surf*. 2012;10:2–14.
10. Szallasi A, Cortright DN, Blum CA, Eid SR. The vanilloid receptor TRPV1: 10 years from channel cloning to antagonist proof-of-concept. *Nat Rev Drug Discov*. 2007;6:357–372.
11. Pan Z, Wang Z, Yang H, Zhang F, Reinach PS. TRPV1 activation is required for hypertonicity-stimulated inflammatory cytokine release in human corneal epithelial cells. *Invest Ophthalmol Vis Sci*. 2011;52:485–493.
12. Pan Z, Capó-Aponte JE, Reinach PS. Transient receptor potential (TRP) channels in the eye. In: Rumelt S, ed. *Advances in Ophthalmology*. Rijeka Croatia: InTech; 2012:35–46.
13. Moreno-Montanes J, Sádaba B, Ruz V, et al. Phase I clinical trial of SYL040012, a small interfering RNA targeting beta-adrenergic receptor 2, for lowering intraocular pressure. *Mol Ther*. 2014;22:226–232.
14. Pecot CV, Calin GA, Coleman RL, Lopez-Berestein G, Sood AK. RNA interference in the clinic: challenges and future directions. *Nat Rev Cancer*. 2011;11:59–67.
15. Coelho T, Adams D, Silva A, et al. Safety and efficacy of RNAi therapy for transthyretin amyloidosis. *N Engl J Med*. 2013;369:819–829.
16. Sheppard JD, Torkildsen GL, Lonsdale JD, et al. Lifitegrast ophthalmic solution 5.0% for treatment of dry eye disease: results of the OPUS-1 phase 3 study. *Ophthalmology*. 2014;121:475–483.
17. 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*. 2000;107:631–639.
18. Tauber J, Karpecki P, Latkany R, et al. Lifitegrast ophthalmic solution 5.0% versus placebo for treatment of dry eye disease: results of the Randomized Phase III OPUS-2 Study. *Ophthalmology*. 2015;122:2423–2431.
19. Kinoshita S, Oshiden K, Awamura S, et al. A randomized, multicenter phase 3 study comparing 2% rebamipide (OPC-12759) with 0.1% sodium hyaluronate in the treatment of dry eye. *Ophthalmology*. 2013;120:1158–1165.
20. Meerovitch K, Torkildsen G, Lonsdale J, et al. Safety and efficacy of MIM-D3 ophthalmic solutions in a randomized placebo-controlled Phase 2 clinical trial in patients with dry eye. *Clin Ophthalmol*. 2013;7:1275–1285.
21. Petrov A, Perekhvatova N, Skulachev M, Stein L, Ousler G. SkQ1 Ophthalmic solution for dry eye treatment: results of a phase 2 safety and efficacy clinical study in the Environment and During Challenge in the Controlled Adverse Environment Model. *Adv Ther*. 2016;33:96–115.
22. Stevenson D, Tauber J, Reis BL; The Cyclosporin A Phase 2 Study Group. Efficacy and safety of cyclosporin A ophthalmic emulsion in the treatment of moderate-to-severe dry eye disease: a dose-ranging, randomized trial. *Ophthalmology*. 2000;107:967–974.
23. Mohapatra DP, Nau C. Regulation of Ca2+-dependent desensitization in the vanilloid receptor TRPV1 by calcineurin and cAMP-dependent protein kinase. *J Biol Chem*. 2005;280:13424–13432.
24. Okada Y, Reinach PS, Shirai K, et al. TRPV1 involvement in inflammatory tissue fibrosis in mice. *Am J Pathol*. 2011;178:2654–2664.