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Aganirsen Antisense Oligonucleotide Eye Drops Inhibit Keratitis-Induced Corneal Neovascularization and Reduce Need for Transplantation

The I-CAN Study

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Objective: Eye drops of aganirsen, an antisense oligonucleotide preventing insulin receptor substrate-1 expression, inhibited corneal neovascularization in a previous dose-finding phase II study. We aimed to confirm these results in a phase III study and investigated a potential clinical benefit on visual acuity (VA), quality of life (QoL), and need for transplantation.

Design: Multicenter, double-masked, randomized, placebo-controlled phase III study.

Participants: Analysis of 69 patients with keratitis-related progressive corneal neovascularization randomized to aganirsen (34 patients) or placebo (35 patients). Patients applied aganirsen eye drops (86 μg/day/eye) or placebo twice daily for 90 days and were followed up to day 180.

Main Outcome Measures: The primary end point was VA. Secondary end points included area of pathologic corneal neovascularization, need for transplantation, risk of graft rejection, and QoL.

Results: Although no significant differences in VA scores between groups were observed, aganirsen significantly reduced the relative corneal neovascularization area after 90 days by 26.20% ($P = 0.014$). This improvement persisted after 180 days (26.67%, $P = 0.012$). Aganirsen tended to lower the transplantation need in the intent-to-treat (ITT) population at day 180 ($P = 0.087$). In patients with viral keratitis and central neovascularization, a significant reduction in transplantation need was achieved ($P = 0.048$). No significant differences between groups were observed in the risk of graft rejection. However, aganirsen tended to decrease this risk in patients with traumatic/viral keratitis ($P = 0.162$) at day 90. The QoL analyses revealed a significant improvement with aganirsen in composite and near activity subscores ($P = 0.039$ and 0.026, respectively) at day 90 in the per protocol population. Ocular and treatment-related treatment-emergent adverse events (TEAEs) were reported in a lower percentage with aganirsen compared with placebo. Only 3 serious TEAEs (2 with aganirsen and 1 with placebo) were considered treatment-related.

Conclusions: This first phase III study on a topical inhibitor of corneal angiogenesis showed that aganirsen eye drops significantly inhibited corneal neovascularization in patients with keratitis. The need for transplantation was significantly reduced in patients with viral keratitis and central neovascularization. Topical application of aganirsen was safe and well tolerated. *Ophthalmology 2014;121:1683-1692 © 2014 by the American Academy of Ophthalmology. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/3.0/).*

Penetrating corneal transplantation has a relatively high success rate due to conditions that favor anterior chamber–associated immune deviation and corneal immune privilege.[1] Multiple mechanisms maintain this privilege, including the blood–eye barrier, the lack of blood and lymphatic vessels in the normal cornea (corneal angiogenic privilege), the relative paucity of mature antigen-presenting cells in the central cornea, the presence of immunomodulatory factors in ocular fluids, and the constitutive expression of CD95L (Fas ligand) within the eye.[2] However, the principal anatomic and biological feature of the cornea determining its immunologic privilege is its avascularity,[2,3] but this privilege can be eroded by inflammation and trauma.[4]

Pathologic corneal angiogenesis in vascularized high-risk beds exposes the graft to immune effector cells, and

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accompanying lymphangiogenesis facilitates the passage of antigen-presenting cells and antigenic material from the graft to regional lymph nodes, inducing alloimmunization and subsequently high rates of graft rejection.[2,5] A recent meta-analysis confirmed the known association between the presence of pathologic corneal neovessels and the increased risk of graft rejection. In fact, the risk of graft rejection increased with the number of corneal quadrants being affected by corneal neovessels.[6,7] Consequently, an expert panel recently declared a high unmet medical need for a topical inhibitor of corneal angiogenesis.[8]

Engagement of insulin receptor substrate-1 (IRS-1) proteins has been shown to be a crucial step in the mechanism leading to angiogenesis,[9] and overexpression of IRS-1 has been found in corneal neovascularization.[10] Aganirsen (GS-101) is an antisense oligonucleotide that inhibits IRS-1 mRNA expression. In preclinical studies, it dose-dependently inhibited IRS-1 expression and in vitro angiogenesis.[9,11] There was also a reduction in vascular endothelial growth factor (VEGF)-A and the proinflammatory cytokine interleukin-1β mRNA expression.[10] Aganirsen has an Orphan Drug designation from the European Regulatory Authorities for the prevention of corneal graft rejection via the management of corneal neovascularization, a well-established risk factor for rejection.[12] Administered as a painless topical application, aganirsen represents a new strategy for inhibition and regression of active ocular angiogenesis. The interim analysis of a phase IIb study previously showed that there was a decrease of 23% in the neovascular area in patients receiving aganirsen at 86 μg/day ($P = 0.0047$) compared with placebo.[13] Although not subjected to a similar interim analysis, the mid-treatment data on visual acuity (VA) suggested an improvement in patients receiving active treatment.[13]

The phase III clinical study reported in this article (the I-CAN [Inhibition of CorneAl Neovascularization] study) is the first phase III study on a topical antiangiogenic agent for use at the ocular surface and the cornea. It was primarily aimed at confirming the encouraging results seen for aganirsen on corneal neovascularization in the phase II analysis. It also investigated the effect of a topical angiogenic inhibitor on VA, need for subsequent transplantation, and quality of life (QoL). One underlying hypothesis was to examine whether an antiangiogenic therapy concomitant to an antiviral/antibacterial therapy (used as primary prevention) could influence or even decrease the need for subsequent corneal transplantation. This would be a novel therapeutic concept and would have great socioeconomic and medical implications.

Methods

Study Objectives

The overall objectives were to first confirm the inhibitory effect of aganirsen on corneal neovascularization in a placebo-controlled study in patients who have keratitis or keratouveitis of bacterial, viral, or traumatic origin, and potentially requiring a corneal transplantation. This study also aimed at investigating a possible correlation with VA as the main clinical benefit. Other clinical benefits were investigated, including QoL, need for transplantation, and risk of graft rejection after potential future transplantation. The safety and tolerability of aganirsen also were assessed.

Patients

Included patients had keratitis or keratouveitis of bacterial, viral (e.g., herpes), or traumatic (e.g., alkali burns) origin. To be eligible for inclusion, patients had to show evidence of progressive stromal neovascularization documented during a minimum period of 1 week (1 month for patients recruited in French centers) and a maximum of 2 months with corneal vessels reaching out for at least 2.5 mm from the limbus or reaching the corneal center. This was assessed using a standardized semiquantitative method on at least 2 photographs of the cornea and validated by a Centralized Reading Center (Felix Bock and Claus Cursiefen, Corneal Angiogenesis Laboratory, University Erlangen-Nürnberg, Germany).[14] At inclusion, participants also had a VA score >20/200 and <20/20.

Institutional review board/ethics committee approvals were obtained for each of the participating centers. Patients provided written informed consent, and the study was performed according to the Declaration of Helsinki and the International Conference on Harmonization Good Clinical Practice guidelines. This study was registered under the following number in the EudraCT database: 2008-005388-33, with the protocol code number GS101-P3-CG.

Study Design

This multicenter, double-masked, randomized study compared aganirsen eye drops with placebo in 2 parallel groups and was performed in 10 centers in 3 European countries (Germany, Switzerland, and France). After validation of progressive corneal neovascularization by the Centralized Reading Center, 2 subgroups of patients were predefined: patients with central neovascularization (i.e., with vascularization extending beyond 2.5 mm from the limbal arcade and reaching the central 4 mm of the cornea) and patients with peripheral neovascularization (i.e., with vascularization extending beyond 2.5 mm from the limbal arcade, but not extending into the central 4 mm of the cornea). The neovascularization status was used as a stratification factor when randomizing patients to the treatment (aganirsen or placebo) groups.

Randomized patients were instructed to apply 1 drop of aganirsen at 0.86 mg/ml (43 μg/drop, i.e., 86 μg/day) or placebo (0.9% saline) in the affected eye twice daily (in the morning and evening) for 90 days. In cases of bilateral lesions, only the worst affected eye was treated. In this study, concomitant medications were avoided, but investigators were permitted to prescribe treatments as deemed necessary. Although other antiangiogenic treatments were prohibited during the study, patients were allowed to continue with topical steroids, antibiotics, acyclovir, or cyclosporine as long as the dose remained stable for the study duration and was in accordance with center practice and local guidelines.

Efficacy and safety variables were assessed at day 1 (baseline), day 30, day 90 (end of study treatment), and day 180 (follow-up). For patients recruited in French centers, an extra visit was performed at day 7 for assessing safety.

Efficacy Assessments

As stipulated by the Regulatory Authorities, the primary efficacy end point was VA at day 90, and this variable was assessed using the standardized Early Treatment Diabetic Retinopathy Study method.[15–17] The secondary end points included the relative and absolute changes in area of corneal neovascularization from baseline (the relative change was the main efficacy criterion for this end point), the extent of neovascularization (by corneal quadrant for stromal or epithelial vessels), the QoL evaluation, the need for

transplantation, and the risk of graft rejection after potential future transplantation, all evaluated at day 90.

As mentioned for the patients' inclusion, measurements of the area of corneal neovascularization were evaluated by the standardized semiquantitative method of computerized morphometry.[14] The need for transplantation was assessed by rating the index of need for penetrating keratoplasty as high, medium, or low, and the risk of graft rejection after potential future transplantation was evaluated following a risk profile established according to the Collaborative Corneal Transplantation Study data[18] and Australian Graft Registry.[19] The QoL was assessed using the National Eye Institute Visual Functioning Questionnaire-25 (Version 2000).[20,21] These efficacy criteria also were evaluated at day 180, and efficacy was assessed at day 90 and day 180 after ocular examination: This examination included intraocular pressure measurement; examination of ocular tissues, such as cornea, iris, lens, and vitreous; evaluation of corneal sensitivity using the noncontact corneal aesthesiometer method; and assessment of the presence of ulcerations and punctuations after fluorescein staining.

Patient compliance was assessed by the number of used and unused single doses of study treatment in relation to the duration of treatment. In addition, compliance with using at least 1 vial per day was considered. The patient was considered noncompliant if the total number of vials used +2 was strictly inferior to the number of days between the first medication administration and the date of visit scheduled at day 90.

Safety Assessments

Adverse events were monitored from the time of informed consent to 3 months after treatment ended. Standard clinical laboratory safety analyses, including hematology, biochemistry, and urinalysis, were performed at day 1 and day 90, and physical examination and vital signs were monitored at all visits. Severity of any tolerability parameters, that is, itching, redness, burning sensation, foreign body sensation, blurred vision, and eye watering, were assessed at all visits by the patient using a Visual Analogue Scale (VAS) ranging from 0 to 100 mm. In addition, the investigator and patient collaborated to score the severity of the symptom according to the following scale: 0 = absent, 1 = mild, 2 = moderate, and 3 = severe.

Image Analysis

Pictures of the cornea were taken for inclusion at visit 1 (prescreening), visit 2 (screening), visit 3 (randomization/inclusion), visit 4 (day 30±3), visit 5 (day 90±7), and visit 6 (day 180±15) at the Centralized Reading Center (Felix Bock and Claus Cursiefen, Corneal Angiogenesis Laboratory, University Erlangen-Nürnberg, Germany). The standardized conditions and technical methods for assessing corneal neovascularization have been described.[14] Blinded analyses of the photographs of the cornea were performed by semiquantitative measurements and computerized morphometry using image analysis software based on grey filter sampling (Cell^f; Olympus Soft Imaging Solutions GmbH, Münster, Germany). Briefly, after contrast and shading optimization of each image, a region of interest was determined along the limbus. The vascularized area (expressed as a percentage) was then calculated by threshold setting and normalized to the total region of interest. Progression in corneal neovascularization was defined as vascularized area at a time point strictly superior to vascularized area at baseline.

Statistical Analysis

All statistical analyses were performed using SAS version 9.3 (SAS Inc, Cary, NC). Comparisons between treatment groups in primary end point and secondary end points were 2-sided, and the significance level was set to $\alpha = 0.050$. The secondary analyses were performed for exploratory purposes. The P values of secondary analyses were not corrected for multiple testing and so should be viewed as nominal.

Chi-square or Fisher exact tests were performed on categoric variables. Logistic models were used for secondary ordered efficacy parameters, including the need for transplantation and risk of graft rejection. The "need for transplantation" was recorded as a binary variable, where 0 = low and 1 = medium, high. The "risk of graft rejection" was recorded as a binary variable, where 0 = low, very low, none, and 1 = medium, high, very high. Odds ratios for need for transplantation and risk of graft rejection calculated via logistic model were adjusted for type of neovascularization (central/peripheral). Baseline value of the binary parameter was added into the model as other covariate. For continuous variables, analysis of covariance (ANCOVA) was performed on efficacy criteria. Evolution of the relative changes in area of neovascularization from baseline to day 180 was analyzed using a mixed-effect model with patient identity as a random effect, with treatment, type of neovascularization (central/peripheral), visit, interaction between visit and treatment, and baseline value as fixed effects.

Five populations were defined: the screened population, the randomized population (all patients randomized to study treatment), the intention-to-treat (ITT) population (all randomized patients who had at least 1 post-baseline VA assessment), the per protocol (PP) population (all ITT patients who did not have any major protocol deviations), and the safety population (all randomized patients who had at least 1 dose of study treatment). The ITT population was the primary efficacy population.

Analyses of 4 efficacy variables (VA, relative change in area of neovascularization from baseline, need for transplantation, and risk of graft rejection) were performed for 2 subgroups of patients. First, the ITT population was subdivided according to the type of neovascularization (i.e., central and peripheral) and the origin of keratitis or keratouveitis (i.e., bacterial, traumatic/viral, and viral).

Results

Study Population

Study participation was from May 2009 to February 2013. Of 228 subjects screened, 99 were included—49 in the aganirsen group and 50 in the placebo group—from 10 centers in Switzerland, France, and Germany (Table 1). Those 99 patients were retained for the safety population, but 30 (15 from each group) were excluded from the ITT population because they did not meet the criterion for evidence of progressive neovascularization and 2 patients were withdrawn after randomization, both from the aganirsen group, because of an adverse event before day 90 (1 patient) and loss to follow-up at day 180 (1 patient). No patients from the placebo group were withdrawn from the study (Table 1). There were a total of 22 major protocol violations leading to exclusion from the PP population: The most common violation was the day 90 visit being performed after day 90±7 or 10 days after the last study treatment.

Patients were well matched in both treatment groups in the ITT and safety populations (Table 2). The most common cause of keratitis or keratouveitis was traumatic in the aganirsen group (44.1%) and viral in the placebo group (57.1%) (Table 2): Comparison of keratitis origin between the treatment groups was close to significance (nominal P value = 0.053, chi-square test). No major differences were found at baseline between the 2 groups in vessel types—epithelial or stromal—in the 4 corneal quadrants (data not shown), VA, need for transplantation, or risk of graft

Table 1. Disposition of Patients and Analysis Sets

	All Randomized Treated Patients N (%)	Patients Using Aganirsen N (%)	Patients Using Placebo N (%)
Included patients	99 (100)	49 (100)	50 (100)
ITT population	69 (69.7)	34 (69.4)	35 (70)
Central neovascularization*	22 (22.2)	11 (22.4)	11 (22)
Peripheral neovascularization†	47 (47.5)	23 (46.9)	24 (48)
Randomized patients withdrawn from the study	2 (2)	2 (4.1)	-
AE	1 (1)	1 (1)	-
Lost to follow-up	1 (1)	1 (1)	-
PP population	47 (47.5)	19 (38.8)	28 (56)
Safety population	99 (100)	49 (100)	50 (100)

AE = adverse event; ITT = intention-to-treat; PP = per protocol.
*Defined as vascularization extending beyond 2.5 mm from the limbal arcade and reaching the central 4 mm of the cornea.
†Defined as vascularization extending beyond 2.5 mm from the limbal arcade, but not extending into the central 4 mm of the cornea.

rejection (Table 2). Compliance with study treatment was high, with 95.9% in the aganirsen group and 100.0% in the placebo group using at least 1 vial per day (data not shown).

Primary Efficacy End Point: Visual Acuity

Aganirsen did not statistically improve absolute VA value at day 90 compared with placebo in the ITT population (Table 3). Similar results were obtained for the PP population. Statistical analysis showed no influence of the following 4 factors—type of neovascularization (central/peripheral), study center, age at baseline, and gender—on the absolute VA values at day 90 (data not shown).

Further analyses of this end point were performed on subgroups according to the origin of keratitis (bacterial, traumatic/viral, and viral) and the type of neovascularization (central and peripheral). No significant effect of aganirsen was observed on VA in the subgroups (data not shown).

Secondary Efficacy End Points

Corneal Neovascularization. The relative change in area of corneal neovascularization from baseline to day 90 was the main secondary efficacy end point. Aganirsen significantly improved the relative area of corneal neovascularization after 3 months of treatment (day 90) at a daily dose of 86 μg: a significant improvement of 26.0% was observed between baseline and day 90 with aganirsen compared with placebo (nominal P value = 0.041) (Fig 1, Table 4). Furthermore, a significant improvement of 26.20% in the relative area of corneal neovascularization also

Table 2. Patient Demographics and Baseline Characteristics in Intention-to-Treat Population

	All Patients N = 69	Aganirsen N = 34	Placebo N = 35
Age (yrs)			
Mean (SD)	51.3 (14.08)	48.7 (15.98)	53.8 (11.63)
Median	52.0	49.5	54.0
Min; max	18; 81	18; 81	26; 75
Sex			
Female (%)	26 (37.7)	13 (38.2)	13 (37.1)
Male (%)	43 (62.3)	21 (61.8)	22 (62.9)
Ethnicity			
Caucasian (%)	69 (100.0)	34 (100.0)	35 (100.0)
Origin of keratitis or keratouveitis*			
Traumatic (%)	27 (39.1)	15 (44.1)	12 (34.3)
Bacteria and other microorganisms (%)	13 (18.8)	9 (26.5)	4 (11.4)
Viral (herpetic) (%)	30 (43.5)	10 (29.4)	20 (57.1)
Need for transplantation†			
High/medium (%)	36 (52.1)	16 (47.0)	20 (57.1)
Low (%)	33 (47.8)	18 (52.9)	15 (42.9)
Risk of graft rejection†			
Medium/high/very high	45 (65.2)	22 (64.7)	23 (65.7)
None/very low/low	24 (34.8)	12 (35.3)	12 (34.3)
VA			
Mean (SD)	53.67 (22.656)	55.32 (21.990)	52.06 (23.492)
Median	56.00	58.00	52.00
Min; max	10.0; 90.0	12.0; 85.0	10.0; 90.0

ITT = intention-to-treat; SD = standard deviation; VA = visual acuity.
*One patient had >1 origin of keratitis or keratouveitis.
†As assessed by the investigators.

Table 3. Absolute Visual Acuity Values at Day 90 in Intention-to-Treat Population

	All Patients N = 69	Aganirsen N = 34	Placebo N = 35
Absolute value			
Mean (SD)	53.43 (±26.851)	55.06 (±25.605)	51.86 (±28.291)
Median	58.00	55.50	58.00
Min; max	0.0; 100.0	0.0; 100.0	0.0; 92.0
ANCOVA, $P = 0.982$			
Estimated value		54.30	54.21
95% CI		48.09–60.51	47.99–60.42

ANCOVA = analysis of covariance; CI = confidence interval; SD = standard deviation.

was observed with aganirsen compared with placebo at day 180 (nominal P value = 0.016), indicating a persisting inhibitory effect of aganirsen 3 months after treatment discontinuation (Fig 1, Table 4). The results were not influenced by the baseline data or the type of neovascularization (peripheral or central) (data not shown). Further analysis of the relative area of corneal neovascularization using a mixed-effect model showed differences of 26.20% at day 90 (nominal P value = 0.014) and 26.67% at day 180 (nominal P value = 0.012), in favor of aganirsen (data not shown).

Statistical analyses by ANCOVA of the absolute changes in area of corneal neovascularization from baseline showed a difference of 1.12% (95% confidence interval [CI], −2.46 to 0.23) in favor of aganirsen (nominal P value = 0.143) at day 90 (data not shown). However, the comparison of this secondary end point between treatments was close to significance at day 180: a difference of 1.14% (95% CI, −2.37 to 0.10) in favor of aganirsen with nominal P value = 0.050 was observed at this time point (data not shown).

Further analyses by subgroups (origin of keratitis and type of neovascularization) confirmed the results obtained in the overall ITT population: in each analyzed subgroup, there was a significant reduction (nominal P value <0.05) in the relative area of corneal neovascularization with aganirsen at both day 90 and day 180 (data not shown).

Extent of Neovascularization. No relevant differences between aganirsen and placebo treatments in the extent of epithelial vessels were observed in any corneal quadrants (data not shown). For the extent of stromal vessels, no relevant differences between treatments were observed in the nasal inferior or the temporal quadrant (data not shown). However, an ANCOVA analysis at day 90 showed a significant difference of 1.56 mm (95% CI, 0.21−2.91) in favor of placebo in the nasal superior quadrant (nominal P value = 0.021, data not shown), although the analysis was performed on imbalanced groups because of missing data (9 patients in the aganirsen group and 20 patients in the placebo group).

Need for Transplantation. No relevant differences between the aganirsen and placebo groups were observed in the need for transplantation at day 90 in the overall ITT (Table 5). However, at day 180, this difference was close to significance (nominal P value = 0.087, chi-square test). The percentage of patients with a low need for transplantation was higher in the aganirsen group than in the placebo group (Table 5).

Further analyses showed that in the subgroup with viral origin of keratitis, the percentage of patients with a low need for transplantation at day 180 was numerically higher in the aganirsen group than in the placebo group (nominal P value = 0.121, chi-square test) (Table 6). Similar results were obtained in the subgroup of patients with central neovascularization (nominal P value = 0.170, chi-square test) (Table 6). When analyzing the need for transplantation in patients with keratitis of viral origin and with central neovascularization, results showed that aganirsen treatment significantly decreased the need for transplantation at both day 90 (nominal P value = 0.048, data not shown) and day 180 (nominal P value = 0.048, Fisher exact test) (Table 6).

Risk of Graft Rejection. No relevant differences between the aganirsen and placebo groups were observed in the risk of graft rejection at day 90 or day 180 in the overall ITT population (nominal P values were 0.346 and 0.482, respectively, chi-square test) (Table 5). However, by using a logistic model, the influence of the type of neovascularization (central or peripheral) on risk of rejection (high/medium vs. low) at day 90 in the ITT population was close to significance (nominal P value = 0.072, data not shown).

Analysis of the subgroup of patients with keratitis of traumatic/viral origin revealed a trend toward a lower risk of graft rejection at day 90 in favor of aganirsen (nominal P value = 0.162, chi-square

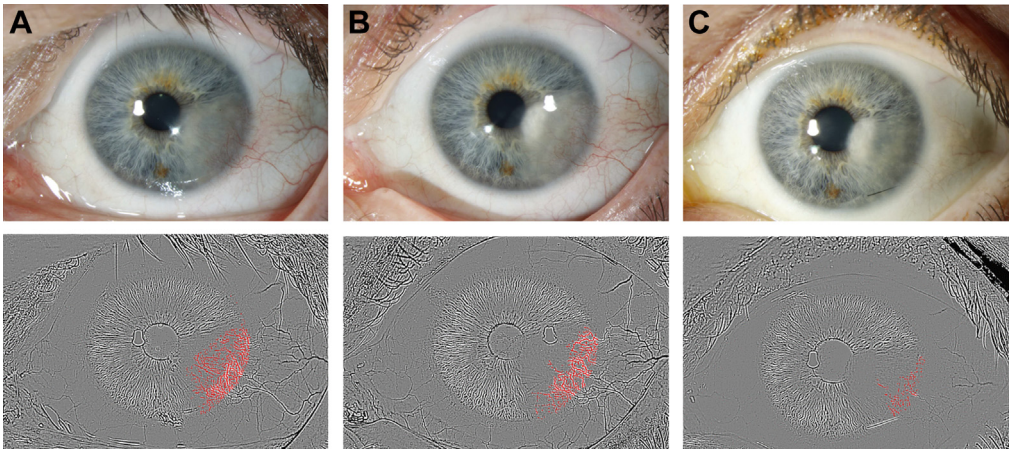

Figure 1. Typical photographs (*top*) with corresponding morphometric analyses (*bottom*) of a patient with progressive corneal neovascularization due to herpetic viral keratitis before treatment **(A)** and after 90 days (**B**) and 180 days (**C**) of aganirsen treatment. Note the significant reduction in area covered by pathologic corneal neovessels.

Table 4. Relative Changes in Area of Corneal Neovascularization at Day 90 and Day 180 in Intention-to-Treat Population

	All Patients N = 69	Aganirsen N = 34	Placebo N = 35
Relative change from baseline at day 90 (%)			
N	68	33	35
Mean (SD)	3.84 (47.849)	−9.62 (29.291)	16.54 (57.984)
Median	−0.67	−6.77	7.06
Min; max	−81.7; 189.7	−76.4; 50.3	−81.7; 189.7
ANCOVA, nominal $P = 0.041$			
Estimated relative change at day 90 (%)		−11.48	14.52
95% CI		−28.35 to 5.40	−1.79 to 30.33
Relative change from baseline at day 180 (%)			
N	68	33	35
Mean (SD)	2.82 (45.265)	−10.88 (35.664)	15.75 (49.862)
Median	−3.73	−16.58	14.11
Min; max	−87.3; 137.0	−81.7; 66.3	−87.3; 137.0
ANCOVA, nominal $P = 0.016$			
Estimated relative change at day 180 (%)		−10.36	15.84
95% CI		−26.21 to 5.48	0.52−31.15

ANCOVA = analysis of covariance; CI = confidence interval; SD = standard deviation.

test) (Table 7). This effect of aganirsen also was observed at day 90 in the subgroup of patients with central neovascularization (nominal P value = 0.201, chi-square test) (Table 7).

Quality of Life. The National Eye Institute Visual Functioning Questionnaire-25 questionnaire used to evaluate the effect on QoL consisted of an overall composite score and 12 subscores assessing the following aspects: general health, general vision, ocular pain, near activities, distant activities, social functioning, mental health, role difficulties, dependency, driving, color vision, and peripheral vision. Although no relevant differences between aganirsen and placebo groups were observed in the QoL questionnaire composite score at day 90 in the ITT population, the PP population showed after ANCOVA analysis a significant improvement of 2.21 (95% CI, −2.74 to 7.16) in the composite score with aganirsen at day 90 compared with placebo (nominal P value = 0.039, data not shown).

Analyses of QoL subscores in the PP population showed a significant improvement of 9.96 (95% CI, 3.00−16.91) in near activities visual function with aganirsen compared with placebo at day 90 (nominal P value = 0.026), although this was not confirmed in the ITT population (data not shown). No relevant differences between the groups were observed in composite score or subscores of the QoL questionnaire at day 180 in the ITT population.

Complete Ophthalmic Evaluation. No relevant differences were observed between the 2 groups in the absolute values of intraocular pressure; corneal sensitivity; presence of ulcerations or punctuations after fluorescein staining analysis; status of lens, iris, and vitreous; or the presence of cataracts at day 90 and day 180 in the ITT population (data not shown).

Safety. Similar numbers of treatment-emergent adverse events (TEAEs) were observed in both groups, and of these, ocular TEAEs considered treatment-related represented 11.1% and 18.4% in the aganirsen and placebo groups, respectively (Table 8). Most TEAEs, including ocular TEAEs, were of mild or moderate severity, and there was no evidence of increased incidence of severe TEAE—ocular or otherwise—in either treatment group.

None of the ocular TEAEs in the treated eye were reported in more than 3 patients in either group. In the aganirsen group, the

Table 5. Frequencies for Need for Transplantation and Risk of Graft Rejection at Day 90 and Day 180 in Intention-to-Treat Population

	All Patients N = 69	Aganirsen N = 34	Placebo N = 35	*P* Values
Need for transplantation				
Day 90				
N	68	33	35	
High/medium (%)	37 (54.4)	17 (51.5)	20 (57.1)	0.641
Low (%)	31 (45.6)	16 (48.5)	15 (42.9)	
Day 180				
N	66	32	34	
High/medium (%)	36 (54.5)	14 (43.8)	22 (64.7)	0.087
Low (%)	30 (45.5)	18 (56.3)	12 (35.3)	
Risk of graft rejection				
Day 90				
N	68	33	35	
Medium/high/very high (%)	45 (66.2)	20 (60.6)	25 (71.4)	0.346
Low/very low/none (%)	23 (33.8)	13 (39.4)	10 (28.6)	
Day 180				
N	66	32	34	
Medium/high/very high (%)	40 (60.6)	18 (56.3)	22 (64.7)	0.482
Low/very low/none (%)	26 (39.4)	14 (43.8)	12 (35.3)	

Table 6. Frequencies for Need for Transplantation at Day 180 in Subgroups

	All Patients N = 69	Aganirsen N = 34	Placebo N = 35	*P* Values
Bacterial origin of keratitis				
N	13	9	4	
High/medium (%)	4 (30.8)	2 (22.2)	2 (50.0)	0.317
Low (%)	9 (69.2)	7 (77.8)	2 (50.0)	
Traumatic/viral origin of keratitis				
N	56	25	31	
High/medium (%)	35 (62.5)	14 (56.0)	21 (67.7)	0.367
Low (%)	21 (37.5)	11 (44.0)	10 (32.3)	
Viral origin of keratitis				
N	30	10	20	
High/medium (%)	15 (50.0)	3 (30.0)	12 (60.0)	0.121
Low (%)	15 (50.0)	7 (70.0)	8 (40.0)	
Central neovascularization				
N	22	11	11	
High/medium (%)	15 (68.2)	6 (54.5)	9 (81.8)	0.170
Low (%)	7 (31.8)	5 (45.5)	2 (18.2)	
Peripheral neovascularization				
N	47	23	24	
High/medium (%)	24 (51.1)	10 (43.5)	14 (58.3)	0.308
Low (%)	23 (48.9)	13 (56.5)	10 (41.7)	
Viral origin of keratitis/central neovascularization				
N	9	4	5	
High/medium (%)	6 (66.7)	1 (25.0)	5 (100.0)	0.048
Low (%)	3 (33.3)	3 (75.0)	-	

most frequently reported ocular TEAEs in the treated eye were ulcerative keratitis and hordeolum (each reported in 2 patients, 4.1%). In the placebo group, they were conjunctivitis (3 patients, 6.0%), ulcerative keratitis, eye allergy, eye irritation, eye pain, eye pruritus, and suture removal (each reported in 2 patients, 4.0%). All the other ocular TEAEs in the treated eye were reported in no more than 1 patient in each group.

Among the treatment-related ocular TEAEs, there was an increased incidence of 2 events—eye pain and eye pruritus—in the placebo group (2 patients, 4.0%, vs. none of the patients treated with aganirsen).

Among the 26 treatment-emergent serious adverse events (SAEs), there were 12 ocular SAEs, which were reported in a lower percentage of patients treated with aganirsen (5 events in 3 patients, 6.1%) than with placebo (7 events in 6 patients, 12.0%). Only 3 treatment-emergent SAEs were considered to be related to treatment: corneal degeneration and corneal disorder, both reported in 1 patient treated with aganirsen, and ocular neoplasm, reported in 1 patient treated with placebo. There were no other significant TEAEs and no deaths.

In regard to ocular tolerability, the highest mean VAS scores (±standard deviation) at baseline were obtained for the item

Table 7. Frequencies for Risk of Graft Rejection at Day 90 in Subgroups

	All Patients N = 69	Aganirsen N = 34	Placebo N = 35	*P* Values
Bacterial origin of keratitis				
N	13	9	4	
Medium/high/very high (%)	4 (30.8)	4 (44.4)	-	0.109
Low/very low/none (%)	9 (69.2)	5 (55.6)	4 (100.0)	
Traumatic/viral origin of keratitis				
N	56	25	31	
Medium/high/very high (%)	41 (73.2)	16 (64.0)	25 (80.6)	0.162
Low/very low/none (%)	15 (26.8)	9 (36.0)	6 (19.4)	
Viral origin of keratitis				
N	30	10	20	
Medium/high/very high (%)	21 (70.0)	6 (60.0)	15 (75.0)	0.398
Low/very low/none (%)	9 (30.0)	4 (40.0)	5 (25.0)	
Central neovascularization				
N	22	11	11	
Medium/high/very high (%)	11 (50.0)	4 (36.4)	7 (63.6)	0.201
Low/very low/none (%)	11 (50.0)	7 (63.6)	4 (36.4)	
Peripheral neovascularization				
N	47	23	24	
Medium/high/very high (%)	34 (72.3%)	16 (69.6)	18 (75.0)	0.677
Low/very low/none (%)	13 (27.7)	7 (30.4)	6 (25.0)	

Table 8. Treatment-Emergent Adverse Events in Safety Population

	All Patients N = 99		Aganirsen N = 49		Placebo N = 50	
	n AEs	*n (%) Patients*	*n AEs*	*n (%) Patients*	*n AEs*	*n (%) Patients*
All TEAEs						
Any TEAE	190	74 (74.7)	90	36 (73.5)	100	38 (76.0)
Any treatment-related TEAE	20	11 (11.1)	7	4 (8.2)	13	7 (14.0)
Any TEAE leading to treatment discontinuation	5	3 (3.0)	3	2 (4.1)	2	1 (2.0)
Any severe TEAE	12	9 (9.1)	6	5 (10.2)	6	4 (8.0)
Any SAE	26	19 (19.2)	15	9 (18.4)	11	10 (20.0)
All ocular TEAEs diagnosed in the treated eye						
Any ocular TEAE	60	39 (39.4)	20	16 (32.7)	40	23 (46.0)
Any treatment-related ocular TEAE	18	11 (11.1)	5	4 (8.2)	13	7 (14.0)
Any ocular TEAE leading to treatment discontinuation	4	2 (2.0)	2	1 (2.0)	2	1 (2.0)
Any severe ocular TEAE	6	6 (6.1)	3	3 (6.1)	3	3 (6.0)
Any ocular SAE	12	9 (9.1)	5	3 (6.1)	7	6 (12.0)

AE = adverse event; SAE = serious adverse event; TEAE = treatment-emergent adverse event.

"blurred vision": 37.9 (±33.6) in the aganirsen group and 46.8 (±31.4) in the placebo group, on a scale of 100 (data not shown). No differences were found between treatments at any time point regarding any of the VAS scores of the ocular tolerability items, except foreign body sensation for which the mean score (±standard deviation) at day 180 was significantly lower in the aganirsen group (11.8±17.6) than in the placebo group (22.3±30.7) (nominal P value = 0.040) (data not shown).

Consistent with these findings, the severity of the symptoms assessed during the study showed no difference between treatments at any time point. No safety concerns were raised after assessment of laboratory safety tests, vital signs, or physical examination.

Discussion

The main aims of this study were (1) to confirm the anti-angiogenic and angio-regressive effect of topical aganirsen treatment on progressive corneal neovascularization seen in the interim analysis of the phase II study[13] and (2) to investigate a potential effect on VA, need for transplantation, and QoL as clinical benefits for the patient. Selection of VA as the primary end point was a requirement from the Regulatory Authorities because it has been used as a measure of efficacy in other ophthalmic studies on pathologies affecting the retina, such as macular degeneration and diabetic retinopathy.[22–24] This was to our knowledge the first time that VA was used as a primary end point in a study that included patients with a pathology affecting the cornea.

In our study, we did not observe a positive effect of aganirsen on VA compared with placebo. This finding was consistent with a recently published systematic review of 11 studies that showed only weak evidence to support the hypothesis that in patients with pathologic corneal neovascularization, a treatment-related reduction of active corneal neovascularization was associated with an increase in VA.[25] Therefore, VA may not be the most appropriate primary end point in a clinical study investigating ocular indications associated with corneal neovascularization.[26] This issue also had been raised by a Consensus Conference on Indications for and Treatment Modalities in Corneal Neovascularization.[8] This weak association may be because pathologic corneal neovessels lower VA not only by their presence within the cornea itself but also by inducing secondary effects in the corneal stroma, including edema, lipid keratopathy, and bleeding.

Confirming previously published results,[13] our study showed that aganirsen significantly reduced the relative area of corneal neovascularization by 26.20% after 3 months of treatment at a daily dose of 86 μg compared with placebo. Furthermore, 3 months after treatment discontinuation, an improvement of 26.67% in the relative area of corneal neovascularization could still be observed in the aganirsen group. The persisting inhibitory effect of aganirsen may be explained by the anti-inflammatory activity of aganirsen, complementing its antiangiogenic effect. Indeed, in preclinical studies, daily aganirsen dosing has been shown to reduce corneal angiogenesis and proinflammatory cytokine production.[9] In ocular pathologies, inflammation and angiogenesis are often associated,[27] and the results suggested that aganirsen could have a dual action on corneal neovascularization.

Several reasons could explain why vision may not have been affected in this study, whereas vascular progression/regression was significantly changed. First, vessels often grow first into the peripheral cornea and so are less likely to affect vision. Second, the secondary changes, such as edema caused by neovessel edema, are unpredictable. Third, there was a great variability in the included disease populations with only limited knowledge on their natural disease course.

In terms of the other secondary end points, aganirsen tended to decrease the need for transplantation in the overall ITT population as assessed by the investigators, although this reduction was not significant (P = 0.515). However, when analyses were performed on patients combining both viral keratitis and central neovascularization, aganirsen significantly decreased the need for transplantation, although these results should be carefully interpreted because of small sample sizes. This could indicate a more favorable outcome in terms of need for transplantation with aganirsen in patients with viral keratitis and central

neovascularization. Further research to confirm these encouraging results is warranted. Nonetheless, for the first time, the controlled study presented raises a new concept in which the need for transplantation can be decreased by combining an antiangiogenic therapy with a conventional disease-specific (e.g., antiviral) therapy at the cornea. However, this new concept of "primary prevention" needs to be studied in greater detail in a more homogenous patient population.

A large body of preclinical evidence suggests herpes viruses to be a prime source of proangiogenic VEGF. In addition, VEGF-mediated angiogenesis has been shown to be an important part of the herpetic keratitis disease process.[28–30] In fact, in an animal model of viral keratitis, antiangiogenic therapy could ameliorate the course of herpetic keratitis disease.[28] This in vivo evidence strongly supports our new concept of antiangiogenic therapy in combination with antiviral therapy in herpetic keratitis being able to alter the disease course in a way that positively affects the need for transplantation.

We did not observe any significant reduction with aganirsen in the assessment of the risk of graft rejection after potential future transplantation. A possible explanation for the lack of efficacy on this end point could be that the "risk of graft rejection" variable was evaluated using a 5-point scale (very high, high, medium, low, very low/none), whereas the "need for transplantation" variable was assessed using a 3-point scale (high, medium, low). This difference would increase the patients' distribution for the "risk of graft rejection" variable and would therefore make the aganirsen effect less marked.

However, we found that the type of neovascularization appeared to influence the risk of rejection, and this effect was close to significance (nominal P value = 0.072). This observation was consistent with a meta-analysis performed on 19 studies: database analysis of 24,944 grafts showed that graft failure and risk of rejection were increased with an increasing number of corneal quadrants affected by neovascularization before keratoplasty.[7]

In our study, the number of randomized patients (n = 99) was relatively low compared with the number of screened patients (n = 228): A total of 129 patients (56.6%) did not meet the inclusion criteria and were excluded. These exclusions probably resulted from the narrow inclusion criteria. First, patients had to have keratitis for which the treatment (including aganirsen) had been given orphan status by European Regulatory Authorities.[12] Second, patients also had to demonstrate progression of neovascularization within the timelines of the study to assess the effect of aganirsen on an active rather than a quiescent corneal neovascular process.

Current treatment of corneal graft rejection is based on steroids and other immune-modulating agents, which are associated with significant side effects. In contrast, this study showed that the safety and tolerability for aganirsen eye drops were comparable to placebo. As such, aganirsen has a promising role in reducing dependency on steroids while significantly improving the rate of success of corneal grafts. Aganirsen has the potential to be the first in its class because there is currently no other trial-tested therapy available that delivers a specific topical antiangiogenic effect at the ocular surface and cornea.

In conclusion, the results of this first phase III study on a topical angiogenesis inhibitor of the ocular surface and cornea showed that aganirsen eye drops significantly inhibited corneal neovascularization in patients with keratitis. The need for transplantation was significantly decreased in the small subgroup of patients with viral keratitis and central corneal neovascularization. Further studies focusing on these patients are necessary to support and confirm the novel concept in which the need for corneal transplantation could be decreased by adding an antiangiogenic therapy to conventional antiviral therapy in herpetic keratitis. Topical application of aganirsen was safe and well tolerated.

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Abbreviations and Acronyms:
ANCOVA = analysis of covariance; **CI** = confidence interval; **IRS-1** = insulin receptor substrate-1; **ITT** = intention-to-treat; **I-CAN** = Inhibition of CorneAl Neovascularization; **PP** = per protocol; **QoL** = quality of life; **SAE** = serious adverse event; **TEAE** = treatment-emergent adverse event; **VA** = visual acuity; **VAS** = visual analog scale; **VEGF** = vascular endothelial growth factor.

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