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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