Development of Noregen™, a novel regenerative ocular therapeutic medicine

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Purpose

The most frequent cause of vision loss & blindness are diseases that disrupt the retinal vasculature (ischemic retinopathies) such as Diabetic Retinopathy and Retinal Vein Occlusion.

Norrin is a Wnt activating growth factor that stabilizes the Brain Retinal Barrier (BRB) & is responsible for the development of the retinal vasculature during development.

For this project, an STTR Phase I grant was obtained to test the feasibility of creating and using Noregen™, a norrin-derived growth factor, to restore proper retinal vasculature in ischemic retinopathies. The project was a partnership between Caeregen Therapeutics (formerly Retinal Solutions) and Oakland University's Eye Research Institute.

A strategy for producing Noregen™ protein in bacteria was developed and tested for toxicity and *in vitro* & *in vivo* efficacy.

Methods

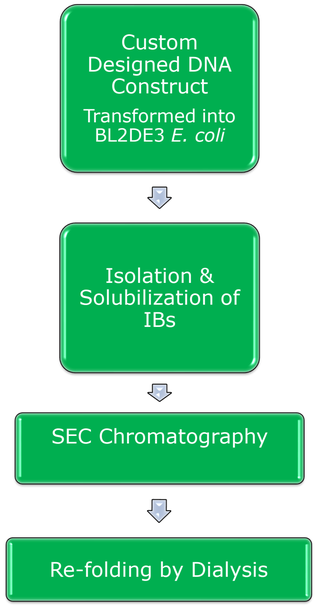
Endotoxin & Receptor (FZD4) binding
- LAL clot assay for Endotoxin
- ELISA: FZD4 coated plates, Serial Noregen dilution, Biotinylated Norrin AB, Streptavidin- HRP detection

ERG & OCT Measurements
- 250 ng Noregen™ IVT injection in 4 Long-Evans Rats
- SD-OCT before IVT injection & at 3 weeks
- Full field ERG at 6 weeks

Proliferation Assay
- Human Retinal Microvascular Endothelial Cells (HRMEC) seeded in 96 well plates, treated with Noregen for 48 hrs, Cell number determined with OrangU dye.

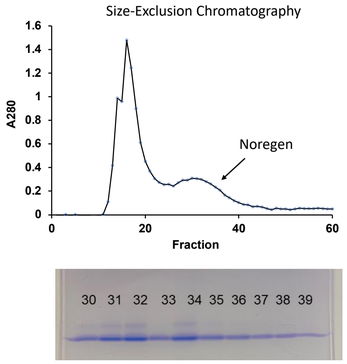
Gene Expression Assays
- Confluent HRMECs treated for 24hrs with 200 ng/ml Noregen™
- RNA isolation & cDNA conversion
- Taqman gene expression assays; *AXIN-2*, *PLVAP* & *TBP* (norm.)

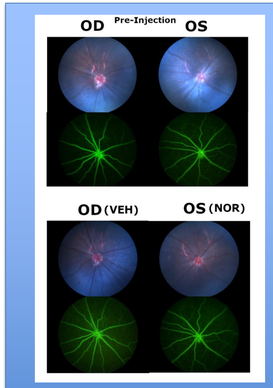
Mouse OIR Model
- C57BL/6 mouse pups in 75% O2 from P7-12
- Noregen (40 ng) IVT in right eyes on P14
- On P17, flat mount retinaes & stain vessels with Isolectin B



Production

- 240 ml culture ⇨ 1.25 g cells
- ~1-gram IB / liter culture
- Endotoxin level; <0.32 EU/ml

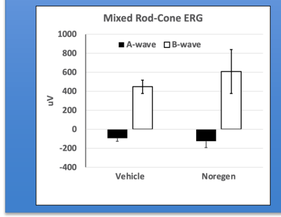




Safety

Fundus images and **FA images (left)** before and 3 weeks after injection of 250 ng Noregen™ in left eyes.

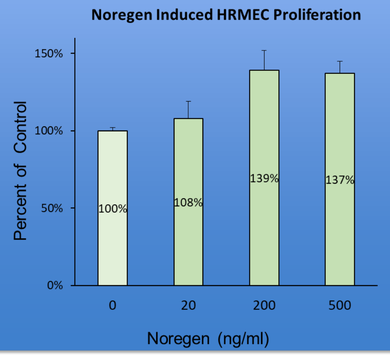
SD-OCT: No differences seen in retinal thickness (Edema), when comparing Noregen™ to Vehicle injected eyes. Retinaes averaged 0.21 mm thickness and over the three-week period the difference in thickness between the OD (vehicle injected) and OS (Noregen injected) eyes never varied more than 1%, confirming the absence of swelling from vascular fluid leakage.

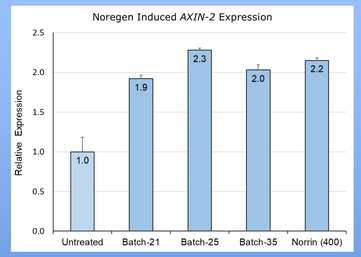


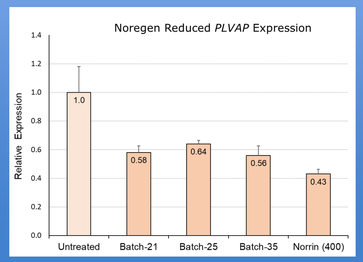
Mixed Rod-Cone **ERG** tests (Left) after Noregen injection. Rod-cone erg amplitudes, 6 weeks after injection. Bars show standard deviation (N-4).

In Vitro (HRMEC) Efficacy Assays

Receptor Binding (EC50's)	
Noregen™ (n=6)	88 ng /ml (+/-33)
Norrin (n=12)	83 ng /ml (+/-34)



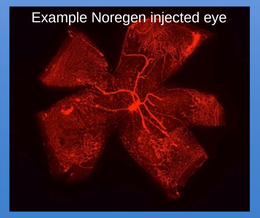




In Vivo (OIR) Efficacy Assay

Relative Retinal Avascular Area	
Noregen (40 ng) Injection	Vehicle Injection
1.015	1.123
0.864	0.955
0.902	1.222
0.845	0.940
0.856	0.978
0.903	

Average relative avascular area in Noregen™ injected OIR eyes was 10% less than in vehicle injected eyes (0.90 vs 1.0). Noregen (n=6), Vehicle (n=5)



Results

- No effects were detected on the microvasculature from injection with Noregen™ in rat eyes.
- Noregen™ did not affect any of the ERG responses compared to vehicle injected control eyes.
- No differences seen in *in vitro* efficacy assays between Noregen™ & Norrin (parent compound)
- Noregen™ treatment at 200 & 500 ng/ml significantly increased the proliferation of HRMECs (p=0.0016 & p=0.0001)
- Noregen™ treatment of HRMECs induced gene expression changes; a 2X increase in Wnt target, *AXIN-2* and a 0.4X decrease in permeability marker, *PLVAP*.
- Noregen™ significantly reduced the Avascular Area in OIR retinas by 10% compared to vehicle injected eyes (p=0.027)

Conclusions

- Noregen™ regenerative potential was demonstrated by an increase in the growth of HRMECs in vitro and an increase in the growth of vessels in vivo (OIR model).
- Noregen™ barrier stabilizing character was demonstrated by a significant reduction in Plasmalemma Vesicle Associated Protein (PLVAP) which is a marker of retinal vascular permeability.
- Noregen™, a novel regenerative ocular therapeutic, was successfully produced in *E. coli* at sufficient scale, purity and biological activity to enable the continued development for future GMP grade manufacturing.

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