Anellovector Derived from Human Retinal Pigment Epithelium Delivers and Expresses a Therapeutically Relevant DNA Payload in the Retina of Nonhuman Primates

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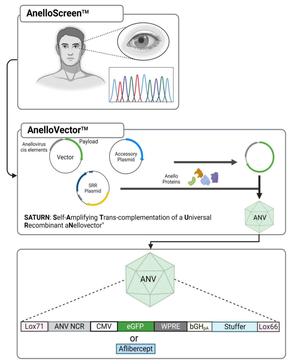
Background

***Figure 1*: Anellogy Platform**



The Anelloviridae family comprises a broad spectrum of viruses found across various animal species, exhibiting significant genetic diversity, which is also observed in humans. These viruses are characterized by their single-stranded circular DNA and possess notable traits such as minimal immunogenicity that makes them a strong vector candidate. We, at Ring Therapeutics, developed the Anellogy platform that screens human anelloviruses from human tissue samples for potential vector-based gene therapy applications. We were successfully able to vectorize an anellovirus derived from human retinal pigment epithelium and demonstrate *in vitro* as well as *in vivo* transduction efficiency. Here, we also highlight the capability of Anellovector for repeated dosing in murine and non-human primate models.

Anellovector transduces RPE cells

- In this experiment we infected iPSC derived RPE cells with ANV.eGFP and performed ISH to demonstrate *in vitro* transduction.
- We observed eGFP genomes in the nucleus within 24 hours through 7 days post-infection. eGFP transcripts were observed and quantified at day 7 and there was significant increase in expression which was validated by eGFP positive cells.

Conclusion:

- This experiment not only provides insights about cellular entry of the vector but also confirms that ANV retains tropism for tissue it originated from.

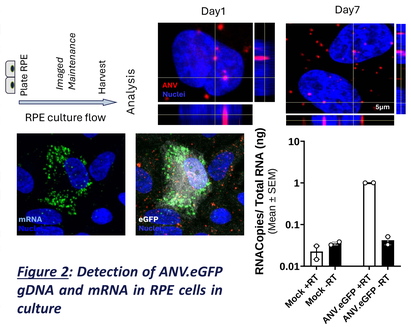


***Figure 2*: Detection of ANV.eGFP gDNA and mRNA in RPE cells in culture**

Anellovector demonstrates transduction in murine eye

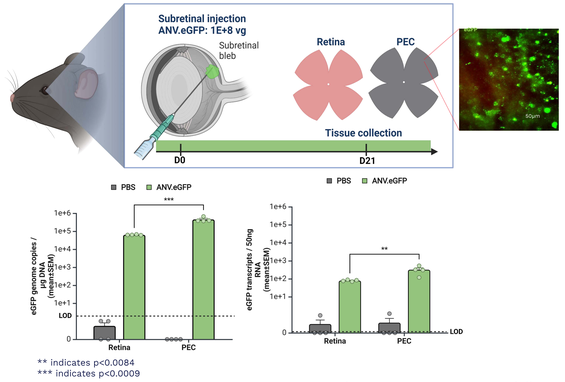


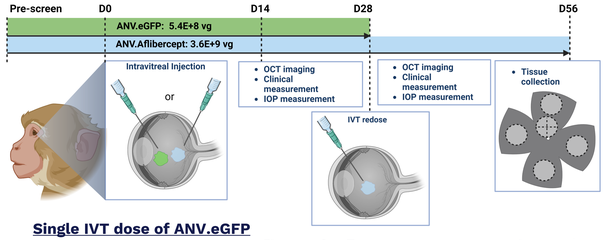
***Figure 3*: Detection of eGFP gDNA, mRNA and protein in murine eye following subretinal administration of ANV.eGFP**

- In this study we wanted to assess transduction efficiency of ANV.eGFP *in vivo*. ANV.eGFP at a dose of 1E+8 vg/eye or PBS was delivered in C57BL/6 mice via subretinal route of administration. Ocular distribution and transgene expression were evaluated at termination on day 21 post treatment.
- Each data point in the plot represents one eye, 10 eyes were processed in each group; of which 4 eyes were processed for DNA and RNA quantification each. 1 eye was dissected for flatmount analysis.
- We observed native eGFP positive cells in the posterior eye cup flatmount.
- The number of eGFP genome copies detected in PEC were significantly more than the neuroretina and similar results were observed for eGFP transcripts as well.
- eGFP genome copies in the negative study control were at or below limit of detection (horizontal dotted line).

Conclusion:

- These results indicate that Anellovector maintains the capacity of the original virus to infect the same tissue it originated from and sustain considerable gene expression for up to three weeks.
- In a separate study, not detailed here, the Anellovector also demonstrated durable *in vivo* function in the mouse eye for 9 months after subretinal administration.
- Based on all the readouts, there was significantly higher expression in the PEC than neuroretina which unlocks the ocular tropism of Anellovector.

Anellovector transduction and redosing in NHP ocular tissues



Single IVT dose of ANV.eGFP or AAV2.eGFP

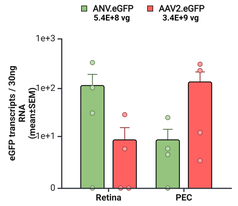
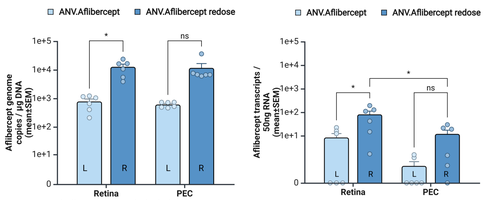


***Figure 4*: Transgene genome copies and transcripts detected in NHP eye following intravitreal administration of Anellovector**

- Following the confirmation of Anellovector transduction in a mouse model, we aimed to assess its transduction capabilities in NHP ocular tissues.
- In this study, we included an additional experimental group to evaluate both, the transduction efficiency of a therapeutic payload and the potential for Anellovector to be re-administered intravitreally.
- In the first study arm, ANV.eGFP was administered intravitreally at a dose of 5.4E+8 vg/eye and study positive control AAV2.eGFP at a dose of 3.4+9 vg/eye. Ocular punches were collected as shown in the study design above. Each data point represents 1 punch. eGFP transcripts were quantified by RT-ddPCR.
- We observed higher expression in the neuroretina from ANV injected eye than AAV2 injected eye. Additionally, we observed expression in the PEC as well.
- In the alternative study arm, ANV.Aflibercept was administered intravitreally at a dose of 3.6E+9 vg/eye on day 0, followed by second dose on day 28.
- Optical Coherence Topography (OCT), Intraocular Pressure (IOP) and Total Clinical Score were determined throughout the study. Transgene expression distribution was evaluated at termination.
- Figure 5 illustrates the analysis of vector infectivity and transgene expression, comparing the outcomes between the eye treated once and that receiving repeated dose.

ANV.Aflibercept Intravitreal Re-administration



Ocular Tolerance to ANV.Aflibercept

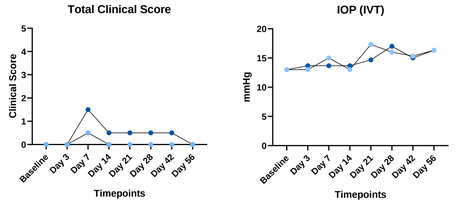


***Figure 5*: Transgene genome copies and transcripts detected in NHP eye following intravitreal re-administration and ocular tolerance to ANV.Aflibercept**

- We observed significant increase in transgene infectivity and expression in the neuroretina of the eye that received repeated doses compared to the eye treated only once.
- Ocular tolerance was accessed by IOP and clinical measurements. No significant adverse events, ocular inflammation, toxicity or ANV- related systemic effect were observed.

Conclusion:

- These results indicate that Anellovector, when administered intravitreally, targets ocular cells and demonstrates higher transgene expression than AAV2 in the neuroretina.
- Anellovector successfully delivered the therapeutically significant payload, Aflibercept, to the retina of non-human primates, showcasing its capability for redosing as well with no signs of ocular toxicity.

Acknowledgements

We would like to thank our internal teams at Ring Therapeutics for their collaboration, guidance, and support at every stage of the project. Additionally, we extend our thanks to Virscio Inc. for conducting the NHP study including injections, pre- and post screening, and ocular imaging.

Oral presentation -
Thurs, May 9, 5:00-5:15 PM ET, Ballroom 4
To learn more about Anellovectors and the Anellogy platform, please attend the oral presentation by Chris Wright, MD, PhD, Chief Medical Officer.

Scan the QR code for details

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