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Preclinical evaluation of NG101 for treatment of wet age-related macular degeneration

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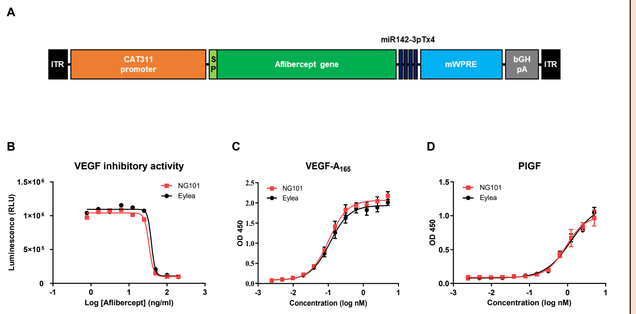
Abstract

Age-related macular degeneration (AMD) is the most common cause of vision loss in individuals over the age of 55, accounting for 6-9% of legal blindness worldwide[1]. A 10-15% of AMD patients develop choroidal neovascularization (CNV) resulting in wet AMD (wAMD), which accounts for almost 90% of blindness associated with AMD[2,3]. We developed NG101 (AAV8.CAT311Aflibercept), a novel gene therapy for the treatment of wAMD using a small promoter, CAT311, derived from the widely-used CAG promoter. This recombinant adeno-associated virus serotype 8 (AAV8) encodes the anti-vascular endothelial growth factor (VEGF) agent, aflibercept. To compare transgene expression between the CAG and CAT311 promoters, mice were administered either AAV8.CAGGFP or AAV8.CAT311GFP via a single subretinal injection. The expression of GFP in the retina was significantly higher with AAV8.CAT311GFP than with AAV8.CAGGFP over a period of 51 weeks. In the laser-induced CNV mouse model, administration of NG101 at doses ranging from $1x10^6$ to $1x10^9$ vg/eye significantly reduced both the leakage and size of CNV lesions in a dose-dependent manner. To evaluate the transgene expression of NG101 in non-human primates (NHPs), cynomolgus monkeys were administered with NG101 via a single subretinal injection with various doses ranging from $3x10^{10}$ to $1x10^{12}$ vg/eye. On day 57, the expression of aflibercept in ocular tissues, including retina, choroid-RPE, vitreous humor (VH), and aqueous humor (AH) exceeded the minimum required levels for effective treatment of wAMD[4]. In addition, cynomolgus monkeys administered with NG101 at a dose of $3x10^{10}$ vg/eye showed a comparable aflibercept expression to cynomolgus monkeys administered with higher doses ($1x10^{11}$ to $1x10^{12}$ vg/eye). Given NG101's robust aflibercept expression capability, we assessed its efficacy at doses of $3x10^9$ and $6x10^9$ vg/eye in the NHP CNV model. Consistent with findings from the mouse CNV study, NG101 effectively inhibited the growth and leakage of new vessels to an extent comparable to the intravitreal injection of Eylea (2mg/eye) in cynomolgus monkeys. Given its potent efficacy at low doses, NG101 is a promising candidate for wAMD treatment, potentially reducing the toxicities commonly associated with AAV-based gene therapies.

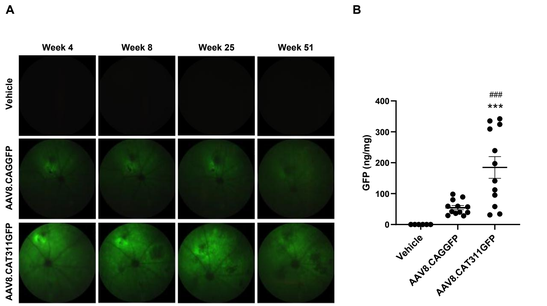
Results

Figure 1. Aflibercept produced by NG101 inhibits VEGF signaling *in vitro*



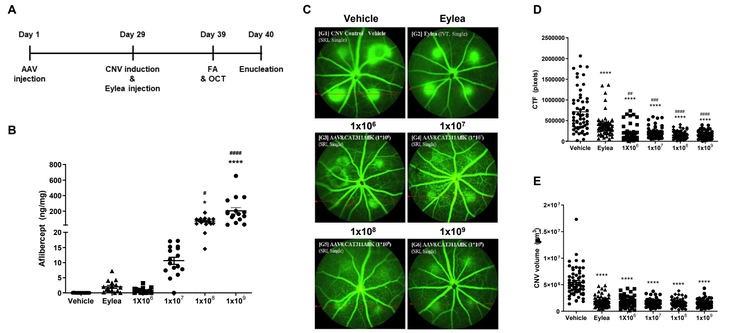
(A) Graphical scheme of aflibercept expression cassette of NG101. (B) The VEGF inhibitory activities of aflibercept produced by NG101 and Eylea® were evaluated. (C and D) The binding selectivity of aflibercept produced by NG101 and Eylea® to recombinant VEGF-A_{165} (C) and placental growth factor (PIGF) (D) were evaluated. RLU: relative luminescence unit. OD450: optical density at absorbance 450nm.

Figure 2. CAT311 promoter drives robust and durable ocular gene expression in mice



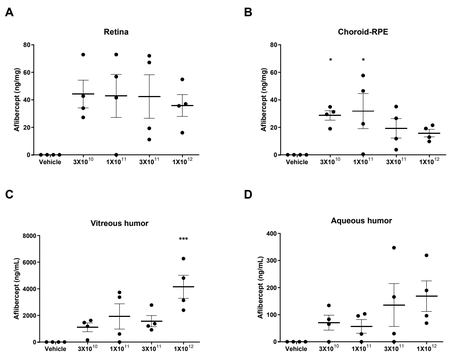
(A and B) Fifty-one weeks after a single subretinal injection of either AAV8.CAGGFP or AAV8.CAT311GFP at a dose of $5x10^8$ vg/eye, GFP expression was evaluated. (A) Representative fundus images are shown. (B) The GFP expression levels were measured. Data are shown as mean ± SEM. (B) Statistical analysis: One-way ANOVA. ***p<0.001 versus Vehicle and ###p<0.01 versus AAV8.CAGGFP.

Figure 3. NG101 administration prevents laser-induced CNV formation in mice



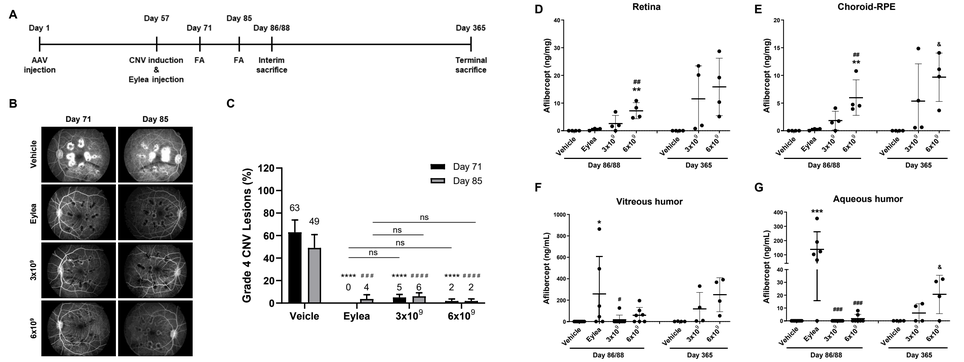
(A-F) Mice were administered NG101 subretinally at indicated doses ($1x10^6$ to $1x10^9$ vg/eye) 28 days prior to CNV induction, or Eylea® (40 μg/μL) intravitreally once immediately after the induction of CNV. (A) Overview of efficacy testing in the mouse model of laser-induced CNV. (B) Ten days post CNV induction, aflibercept expression was measured. Data are shown as mean ± SEM. (C) Representative fluorescein angiography (FA) images are shown. (D) The CNV lesion leakage was evaluated. Data are shown as mean ± SEM. (E) The volume of CNV lesions based on optical coherence tomography (OCT) images was measured. Data are shown as mean ± SEM. (B, D and E) Statistical analysis: One-way ANOVA. *P<0.05 ****P<0.0001 versus Vehicle and #P<0.05, ##p<0.01, ###P<0.001, ####p<0.0001 versus Eylea®. CTF: corrected total fluorescence.

Figure 4. High levels of aflibercept accumulate in the retinal region following a single subretinal administration of NG101 in NHPs.



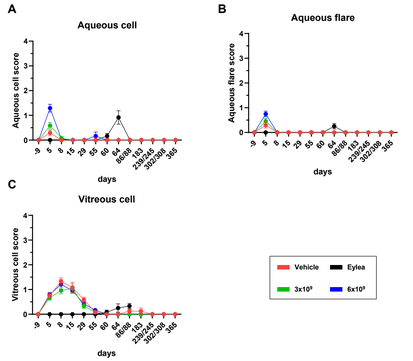
(A-D) Cynomolgus monkeys were administered Vehicle or NG101 at indicated doses ($3x10^{10}$ to $1x10^{12}$ vg/eye) via bilateral subretinal injection. Fifty-seven days post-injection, all animals were euthanized, and aflibercept concentrations were measured in the retina (A), choroid-RPE (B), vitreous humor (C) and aqueous humor (D). Data are shown as mean ± SEM. (A-D) Statistical analysis: One-way ANOVA. *P<0.05, ***P<0.001 versus Vehicle.

Figure 5. Subretinally administered NG101 effectively suppresses CNV formation at low doses in NHPs



(A-G) NG101 was administered subretinally at indicated doses ($3x10^9$ or $6x10^9$ vg/eye) and Eylea® (2 mg/eye) was injected intravitreally in cynomolgus monkeys. (A) Overview of efficacy testing in NHP CNV model. (B) Representative fluorescein angiography (FA) images are shown. (C) The percentage of grade 4 CNV lesions was evaluated. Data are shown as mean ± SEM. (D-G) Aflibercept expression levels in the retina (D), choroid-RPE (E), vitreous humor (F), and aqueous humor (G) were measured at day 86/88. Additionally, two animals (one male and one female) from Vehicle, $3x10^9$, and $6x10^9$ dose groups were followed up until day 365. Data are shown as mean ± SEM. (C-G) Statistical analysis: (C) Two-way ANOVA. ****0.0001 versus Vehicle on day 71 and ###p<0.001, ####p<0.0001 versus Vehicle on day 85. (D-G) Statistical analysis: One-way ANOVA. *P<0.05, **P<0.01, ***P<0.001 versus Vehicle on day 86/88, #P<0.05, ##P<0.01, ###P<0.001 versus Eylea® on day 86/88, $P<0.05 versus Vehicle on day 365, and @P<0.05, @@@P<0.001 versus $3x10^9$ on day 86/88.

Figure 6. Ocular inflammation was mild at the effective doses of NG101 in NHPs



(A-C) Ophthalmic examinations were conducted on the same animals described in Figure 5 to assess ocular inflammatory responses. Parameters evaluated included aqueous cell (A), aqueous flare (B), and vitreous cells (C), monitored up to day 86/88. Additionally, two animals (one male and one female) from each of the Vehicle, $3x10^9$, and $6x10^9$ vg/eye groups were followed up until day 365. Data are shown as mean ± SEM.

Conclusions

- NG101 (AAV8.CAT311Aflibercept) is a recombinant, non-replicating adeno-associated virus 8 (AAV8) expressing aflibercept under the control of CAT311 promoter.
- A single subretinal injection of NG101 prevents laser-induced CNV formation in in mice and NHPs.
- Aflibercept is highly expressed and sustained in the retinal region until day 365 following a single subretinal injection of NG101 in NHPs.
- One day post NG101 administration, the NG101 vector was detected in serum and declined to undetectable levels within a month (Data not shown).
- No or low levels of anti-AAV8 and no anti-aflibercept antibodies were detected in serum and vitreous humor in NHPs (Data not shown).
- There is no NG101-related retinal toxicities in NHPs (Data not shown).
- NG101 is an auspicious therapeutic candidates for wAMD treatment.
- A Phase 1/2a clinical trial for NG101 is currently underway (NCT05984927).

References

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4. Kiss, Szilárd, et al. "Analysis of aflibercept expression in NHPs following intravitreal administration of ADVM-022, a potential gene therapy for nAMD." Molecular Therapy-Methods & Clinical Development 18 (2020): 345-353.

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