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REGENERON

Combination of Intravitreal Aflibercept and Nesvacumab Enhances the Anti-Leak Duration of Aflibercept in a Rabbit Model of Retinal Neovascular Leak

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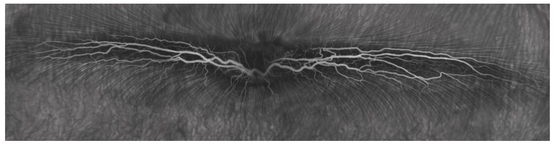
Introduction

- Vascular eye diseases are the leading cause of vision loss in today's aging population, including both exudative age related macular degeneration (Wet AMD) and diabetic retinopathy. Both diseases are characterized by abnormal 'leaky' blood vessels growing into the retina.
- The rabbit model of retinal neovascular leak (RNV) uses a selective glial toxin, DL-alpha-aminoadipic acid (DL-AAA), to induce retinal neovascularization.
- DL-AAA leads to a localized area of retinal degeneration and an altered expression of vascular endothelial growth factor (VEGF).
- Regeneron has produced an anti-VEGF therapy known as aflibercept, which is FDA approved to treat wet AMD, macular edema secondary to central retinal vein occlusion, and diabetic macular edema.
- Nesvacumab is a human monoclonal antibody created using VelocImmune® technology, that binds to and blocks the activity of Angiopoietin2 (Ang2).
- Ang2, along with VEGF, is believed to contribute to pathological angiogenesis and vascular leak in both wet AMD and diabetic eye disease.

Materials and Methods

Normal Rabbit Fundus (FA)



Retinal Neovascularization Induction:

Retinal neovascularization (RNV) was induced by a single 80mM intravitreal (IVT) injection of DL-AAA (80 mcl). The animals received periodic ophthalmic evaluations to monitor the disease progression and any eyes with severe retinal detachment were excluded from the study. Eight weeks following the DL-AAA injection the leak becomes stable. At that point, the animals were segregated into the different treatment groups based on the extent of retinal leak allowing us to obtain homogenous starting populations. The treatment began 10 weeks post DL-AAA administration, following a full ophthalmic examination.

Study 1:

Rabbits were administered a single IVT injection of nesvacumab (500 µg/eye), aflibercept (125 µg/eye), or a co-formulation of aflibercept and nesvacumab (125 µg aflibercept and 500 µg nesvacumab/eye). All rabbits received in-life ophthalmic examinations on weeks 1, 2, 3, 4, 6, 8, 10, 12, and 14. Vascular leak was assessed by fluorescein angiography (FA) during each ophthalmic examination. All animals were euthanized at week 14 post initiation of treatment.

Study 2:

Rabbits were administered a single IVT injection of nesvacumab (500 µg/eye), high-dose aflibercept (500 µg/eye), or a co-formulation of aflibercept and nesvacumab (500 µg aflibercept and 500 µg nesvacumab/eye). All rabbits received in-life ophthalmic examinations on weeks 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 16, 17, 18, 19 and 20. Vascular leak was assessed by fluorescein angiography during each ophthalmic examination. All animals were euthanized at week 22 post initiation of treatment.

Anti-Ang2 Enhances Aflibercept Anti-Leak Effect

Study 1:

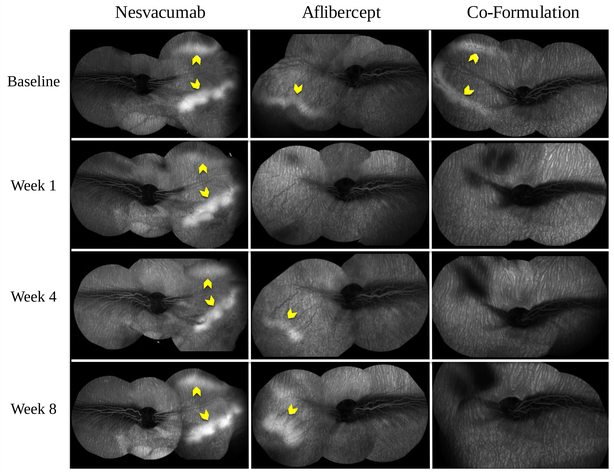


Figure 1: One week after treatment both aflibercept (125 µg/eye) and co-formulation (125 µg aflibercept and 500 µg nesvacumab/eye) showed a significant reduction in vascular permeability. Nesvacumab monotherapy (500 µg/eye) did not appear to have significant effects on vascular permeability. Three weeks after treatment, leakage area was observed in the aflibercept monotherapy cohort, which continued to enlarge in the following weeks. Signs of vascular permeability did not return in the co-formulation cohort until 8 weeks post treatment. Co-formulation had a two-fold increase on duration of effects compared to aflibercept monotherapy.

Study 2:

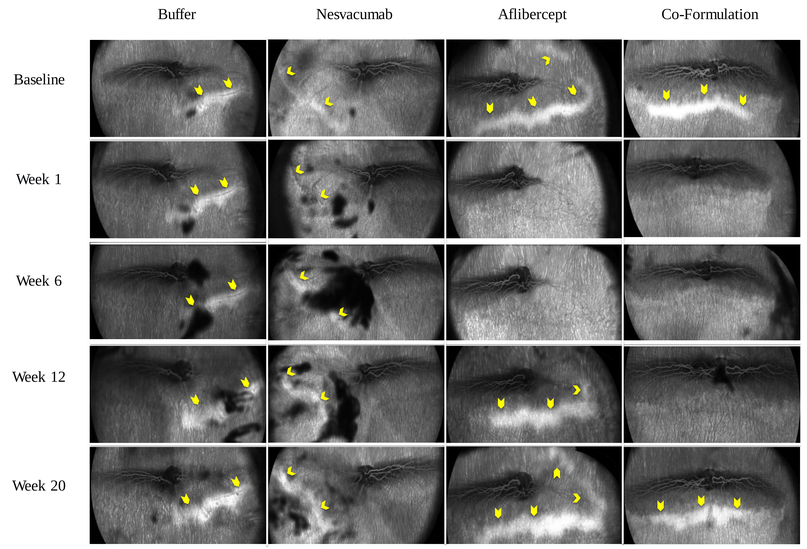


Figure 2: One week after treatment, both aflibercept (500 µg/eye) and co-formulation (500 µg aflibercept and 500 µg nesvacumab/eye) showed a significant reduction in vascular permeability. Buffer (Eylea Buffer) and nesvacumab monotherapy (500 µg/eye) did not completely inhibit vascular permeability, as seen in other cohorts. Eight weeks after treatment, leakage area was observed in aflibercept monotherapy, which continued to enlarge in the following weeks. Signs of vascular permeability did not return in the co-formulation cohort until 9 weeks post treatment with a single eye lasting as long as 20 weeks.

Anti-Ang2 Enhances Aflibercept Anti-Leak Effect *Continued*

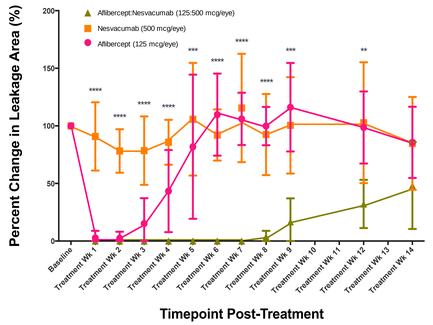


Figure 3: Percent leakage area standardized to baseline leakage area after administration of anti-angiogenic agents. Leakage area is 100% at baseline, immediately before treatment. One week after treatment, both aflibercept and co-formulation (aflibercept:nesvacumab) showed a significant reduction in leakage area. Three weeks after treatment aflibercept monotherapy showed the first sign of recurrent leakage in the FA imaging. The first sign of leakage in the co-formulation treatment group was observed 8 weeks post-treatment. Leakage area had not reached that of baseline area by 14 weeks post-treatment in the co-formulation cohort. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.

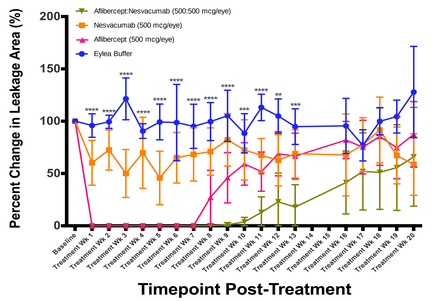
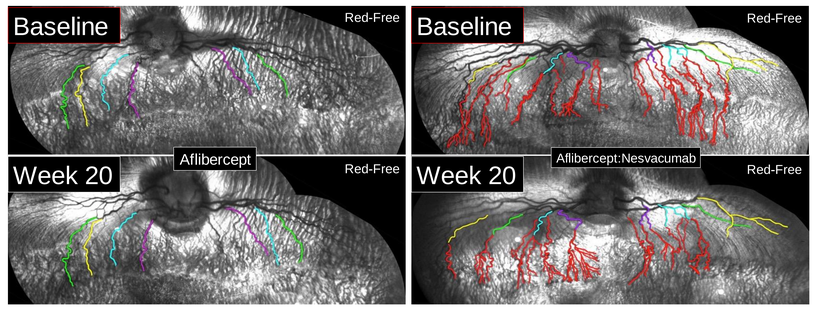


Figure 4: Percent leakage area standardized to baseline leakage area after administration of anti-angiogenic agents. Leakage area is 100% at baseline, immediately before treatment. One week after treatment, both aflibercept and co-formulation (aflibercept:nesvacumab) showed a significant reduction in leakage area. Eight weeks after treatment, high-dose aflibercept monotherapy showed the first sign of recurrent leakage in the FA imaging. The first sign of leakage in the co-formulation treatment group was observed 9 weeks post-treatment. Leakage area had not reached that of baseline area by 20 weeks post-treatment in the co-formulation cohort. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.

Treatment with Co-Formulation Leads to Significant Morphological Differences in Pathological Vasculature Compared to Baseline

Figure 5:



Discussion

IVT administration of nesvacumab monotherapy does not completely inhibit vascular leak after intravitreal administration.

IVT administration of aflibercept completely abolished the retinal vascular leak for a period of approximately 3-5 weeks at low doses and 8-9 weeks at high doses. The vascular leak recurred in the same location observed before treatment and the rate at which leak returned was rapid.

A co-formulation of aflibercept and nesvacumab significantly enhanced the anti-leak effect of aflibercept, whether at low or high doses. The co-formulation inhibited the vascular leak immediately after treatment, similarly to aflibercept monotherapy. However, the anti-leak effect was prolonged and the rate of leakage return was dramatically reduced, possibly due to the growth of new neovessels.

Commercial relationships: E, authors work for Regeneron Pharmaceuticals