VEGF-C and VEGF-D Blockade by VGX-300 Inhibits Choroidal Neovascularization and Leakage in a Mouse Model of Wet AMD

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PURPOSE and METHODS

Choroidal neovascularization (CNV) is the major cause of severe visual loss in subjects with AMD. At least 45% of subjects with wet AMD exhibit some degree of resistance to anti-VEGF-A monotherapy. Resistance to therapy may be related to activity of other proangiogenic factors such as VEGF-C and VEGF-D. VEGF-C and VEGF-D can participate in CNV formation by promoting angiogenesis by binding and activating VEGFR-2 and VEGFR-3. VGX-300 is a soluble form of VEGFR-3 expressed as an Fc-fusion protein that potently binds and inhibits the activity of VEGF-C and VEGF-D but not VEGF-A.

We investigated the efficacy of VGX-300 to inhibit laser-induced CNV formation and vascular leakage and compared it with Eylea® (aflibercept), a 'Trap' for VEGF-A. Laser-induced CNV was created in C57BL/6 mice using a 532 nm laser under direct visualization using a Micron III® fundus camera (4 - 9 spots/eye; 50 µm size, 50 ms, 550 Mw). On day 0 or day 7 post-laser injury, mice were administered a single intravitreal (IVT) injection of a negative isotype antibody control IgG, Eylea®, VGX-300 or the combination of VGX-300 and Eylea®. For each injection, a total of 80 µg protein was administered in a 2 µl injection (for single-agent groups, 40 µg of IgG was added to each 40 µg dose of Eylea® or VGX-300). Extent of leakage and CNV areas were determined by fluorescein angiography followed by intracardiac perfusion of FITC-dextran in gelatin (10%) on day 7 or 14 post-laser burn. Mouse ocular tissue and clinical specimens from AMD patients were histologically evaluated to identify expression and localization of VEGFs and VEGFRs. The modulation of expression of a panel of angiogenesis and inflammatory genes in mouse CNV following VGX-300 administration was evaluated by quantitative RT-PCR (qRT-PCR).

RESULTS
VGX-300 and Eylea® inhibit CNV to a comparable extent in the mouse

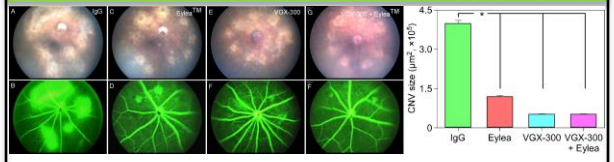

FIGURE 1. Laser injury was induced on day 0 and was followed immediately by injection of IgG, Eylea®, VGX-300 or their combination. Fundus images and angiography were taken on day 14. Both Eylea® and VGX-300 significantly reduced CNV area compared to the IgG control treated group. In this study, the single-agent activity of VGX-300 was highly significant, therefore an additive effect in the VGX-300 + Eylea® group could not be observed in this study. (n=15 mice/group).

Established CNV lesions regress following VGX-300 treatment

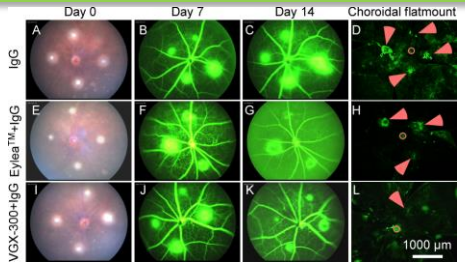

FIGURE 2. Representative fundus images, angiography and choroidal flatmounts of laser-induced CNV membranes 7 and 14 days after laser-burn and administration, on day 7, of **(A – D)** IgG (80 µg), **(E – H)** Eylea® (40 µg) + IgG (40 µg) , or **(I – L)** VGX-300 (40 µg) + IgG (40 µg) (n=10 mice/group). Red circles indicate the optic heads and arrows point to the CNV lesions.

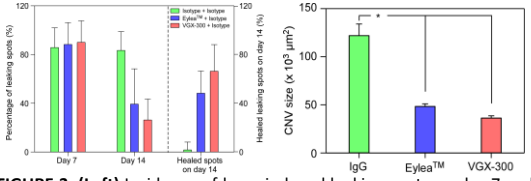

FIGURE 3. (Left) Incidence of laser-induced leaking spots on day 7 and on day 14 post-laser injury (leaking spots/photocoagulated spots × 100%) (n=10 mice/group). Treatments were administered on day 7. **(Right)** Mean size of laser-induced CNV membranes at day 14 following administration of IgG, Eylea® and VGX-300 on day 7 (n=10 mice/group).

Expression of VEGF-C and VEGFR-2/3 in CNV and clinical AMD

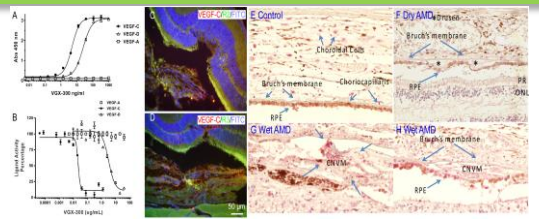

FIGURE 4. (A) VGX-300 binds VEGF-C and VEGF-D but not VEGF-A in a direct binding ELISA and **(B)** VEGFR-2 cell-based bioassay. **(C,D)** Expression of VEGF-C, VEGFR-2 and VEGFR-3 in laser-induced CNV membranes. **(E,F)** Low expression of VEGF-C in control and dry AMD. **(G,H)** Increased expression of VEGF-C around CNV membranes (arrows).

Modulation of gene expression post-laser injury and following VGX-300 treatment

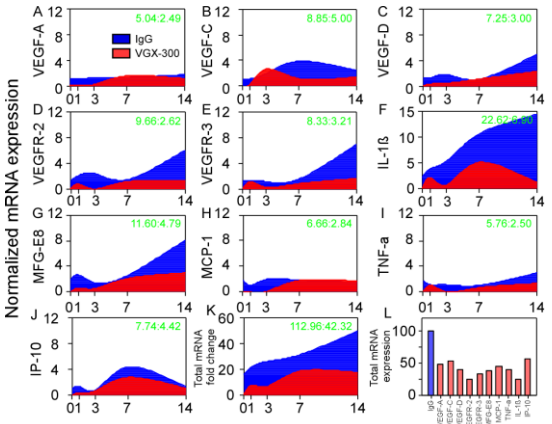

FIGURE 5. (A – J) The dynamic change in mRNA levels of a panel of angiogenic and inflammatory genes on day 0, 1, 3, 7 and 14 post-laser injury in the ocular tissue of mice treated with IgG (**blue**) or VGX-300 (**red**). IgG or VGX-300 was administered on day 0 post-laser injury by IVT injection. The **green** value in each plot is the area ratio of mRNA levels between IgG and VGX-300 treated groups. **(K)** The accumulated expression of this gene panel for each treatment group. **(L)** The normalized accumulated expression of each gene in the VGX-300 compared to IgG group (n=10 mice/group on each sampling day).

CONCLUSIONS

1. VGX-300 mediated blockade of VEGF-C/-D significantly inhibits choroidal neovascularization and vascular leakage comparably to Eylea® in the laser-induced mouse model of wet AMD.

2. Established CNV lesions in the mouse regress following treatment with VGX-300 on day 7 post-laser injury.

3. VEGF-C expression is higher in wet AMD and lower in control and dry AMD clinical specimens.

4. VGX-300 reduces the expression of genes associated with angiogenesis and inflammation following laser-induced injury.

5. Administration of single-agent VGX-300 may be an effective therapy for wet AMD. Administered in combination with anti-VEGF-A therapies, VGX-300 may have the potential to improve clinical responses, particularly in patients that are sub-responsive to anti-VEGF-A therapies.

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