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

Kameran Lashkari1, Jie Ma1, Gianna Teague1, Chenying Guo1, Megan E. Baldwin2
1Schepens Eye Research Institute, Massachusetts Eye & Ear, Department of Ophthalmology, Harvard Medical School, Boston, 02114 MA
2Opthea Pty Ltd, Circadion Technologies, Level 4, 650 Chapel Street, South Yarra, Victoria 3141, Australia

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 VEGF-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 x 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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