SHORT COMMUNICATION

Treatment of Experimental Choroidal Neovascularization via RUNX1 Inhibition

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Choroidal neovascularization (CNV) is a prevalent cause of vision loss in patients with age-related macular degeneration. Runt-related transcription factor 1 (RUNX1) has been identified as an important mediator of aberrant retinal angiogenesis in proliferative diabetic retinopathy and its modulation has proven to be effective in curbing pathologic angiogenesis in experimental oxygen-induced retinopathy. However, its role in CNV remains to be elucidated. This study demonstrates RUNX1 expression in critical cell types involved in a laser-induced model of CNV in mice. Furthermore, the preclinical efficacy of Ro5-3335, a small molecule inhibitor of RUNX1, in experimental CNV is reported. RUNX1 inhibitor Ro5-3335, aflibercept—an FDA-approved vascular endothelial growth factor (VEGF) inhibitor, or a combination of both, were administered by intravitreal injection immediately after laser injury. The CNV area of choroidal flatmounts was evaluated by immunostaining with isolectin B4, and vascular permeability was analyzed by fluorescein angiography. A single intravitreal injection of Ro5-3335 significantly decreased the CNV area 7 days after laser injury, and when combined with aflibercept, reduced vascular leakage more effectively than aflibercept alone. These data suggest that RUNX1 inhibition alone or in combination with anti-VEGF drugs may be a new therapy upon further clinical validation for patients with neovascular age-related macular degeneration. (*Am J Pathol* 2021, 191: 418–424; <https://doi.org/10.1016/j.ajpath.2020.12.005>)

Choroidal neovascularization (CNV) is a major cause of vision loss in a variety of ocular diseases, including neovascular age-related macular degeneration (AMD). AMD is the leading cause of blindness in the elderly in developed countries, and CNV and associated intraretinal fluid account for the majority of visual impairment in AMD.^{1,2}

Current therapy for CNV involves intravitreal injection of drugs that target vascular endothelial growth factor (VEGF).^{3,4} Despite the initial improvement in visual function after treatment with anti-VEGF agents, multiple studies show long-term visual decline.^{5,6} Additionally, patients often require multiple injections in their eyes, and almost half of the patients have persistent retinal fluid despite chronic treatment.^{7,8} These underscore the need to develop new therapeutic strategies for CNV.

Runt-related transcription factor 1 (RUNX1) has been identified by transcriptomic analysis of fibrovascular membranes of patients with proliferative diabetic retinopathy as a regulator of retinal neovascularization.⁹ RUNX1 inhibition results in the reduction of endothelial cell migration,

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L.G.-B. and S.D.-T. contributed equally to this work.

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Figure 1 RUNX1 is expressed in different cell populations involved in CNV formation. **A–E**: Immunofluorescence characterization of RUNX1 in laser-induced CNV with specific markers for endothelial cells labeled with CD31 (**A**), macrophages/microglia labeled with CD11b (**B**), retinal pigment epithelium with RPE65 (**C**), vascular smooth muscle cells/myofibroblasts with α -SMA (**D**), and Müller cells with GFAP (arrows) (**E**). **Boxed areas** are shown at higher magnification to the right. **F**: Section of uninjured retina stained with RUNX1. Scale bars: 100 μ m (**E**, left panel, and **F**); 20 μ m (**E**, right panel). Original magnification: $\times 20$ (**A–D**, left panels); $\times 60$ (**A–D**, right panels) CNV, choroidal neovascularization; GFAP, glial fibrillary acidic protein; SMA, smooth muscle actin.

proliferation, and tube formation *in vitro*, and reduces neovascular area in experimental oxygen-induced retinopathy, without affecting the avascular area.⁹ Based on these data, the authors believe that RUNX1 has a significant effect on pathologic angiogenesis, and that RUNX1 regulation could represent a new therapeutic approach to control the growth of abnormal blood vessels in CNV.

Materials and Methods

Animal Model

Animal procedures were approved by the Institutional Animal Care and Use Committee of Massachusetts Eye and Ear, and performed in accordance with the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research. Six- to 8-weeks-old C57BL/6J male and female mice were purchased from The Jackson Laboratories (Bar Harbor, ME). For all procedures, mice were anesthetized by i.p. injection of a ketamine/xylazine mixture (100/50 mg/kg). Laser photocoagulation was performed under general anesthesia with a 532-nm laser attached to the Micron III image-guide system (Phoenix Technology Group, Pleasanton, CA) using 120 mW power, 50-ms duration, and a spot size of 50 μ m.

Four laser spots were placed around the optic nerve. Twelve laser spots were generated per eye to evaluate mRNA expression. Disruption of Bruch's membrane was confirmed by the appearance of a cavitation bubble. Immediately after laser, a single intravitreal injection was administered with either phosphate-buffered saline (vehicle), aflibercept 10 μ g, Ro5-3335 75 μ mol/L, or a combination of Ro5-3335 75 μ mol/L + aflibercept 10 μ g. Fluorescein angiography was performed 6 days after laser induction by injection of 0.1 mL of 2% sodium fluorescein, and serial photographs were captured. Light source intensity and gain were standardized and maintained in all experiments. Animals were euthanized and samples were collected day 7 post-laser.

Immunostaining of Cryosections

Eyes were enucleated and fixed for 1 hour in 4% paraformaldehyde and then cryoprotected in increasing concentrations of sucrose before embedding in OCT and frozen. Ten μ m sections were thawed, air-dried, incubated in blocking buffer containing 2% bovine serum albumin and 0.3% Triton X-100 for 30 minutes, and subsequently incubated with primary antibody diluted in blocking buffer overnight at 4°C. Primary antibodies used were mouse anti- α -SMA (1:100; Sigma-Aldrich, St. Louis, MO), rat anti-CD11b (1:100; BD

Figure 2 RUNX1 time-course expression. **A:** Representative images of choroidal flatmounts stained with RUNX1 at different time points after CNV induction. **Dotted circles** highlight areas with positive staining for RUNX1. **B:** Quantification of RUNX1 relative fluorescence. **C:** Quantitative RT-PCR analysis of *RUNX1* in retina. **D:** *VEGF* mRNA expression in retina. **E:** Quantitative RT-PCR analysis of *RUNX1* in retinal pigment epithelium (RPE)/choroid. **F:** *VEGF* mRNA expression in RPE/choroid. $n = 6$ mice per group (**B**); $n = 8$ mice per time point (**C–F**). * $P < 0.05$, ** $P < 0.01$. Scale bars = 400 μm . CNV, choroidal neovascularization; VEGF, vascular endothelial growth factor.

Biosciences, San Jose, CA), rat anti-CD31 (1:100; BD Biosciences), goat anti-GFAP (1:100; Abcam, Cambridge, MA), mouse anti-RPE65 (1:100; Abcam), and rabbit anti-RUNX1 (1:100; LifeSpan BioSciences, Seattle, WA). Samples were then incubated with the corresponding secondary antibody for 2 hours at room temperature, and nuclei were stained with DAPI. Finally, samples were mounted with Fluoromount-G (Thermo Fisher Scientific, Waltham, MA). Images were acquired with Leica SP8 confocal microscope (Leica, Wetzlar, Germany).

Choroidal Flatmount Tissue Preparation and Immunostaining

Eyes were enucleated and fixed in 4% paraformaldehyde for 2 hours at 4°C. Cornea, lens, and retina were removed, and eight petals were created by cutting the remaining eyecup. Choroidal flatmounts were blocked with 1% bovine serum albumin, 0.1% Triton X-100, and 3% donkey serum in PBlec buffer overnight at 4°C, incubated with isolectin B4 (IB4) (Thermo Fisher Scientific), rabbit anti-RUNX1 (1:100; LifeSpan BioSciences), or rat anti-CD11b (1:100; BD Biosciences) and the corresponding secondary antibody, and then mounted with Fluoromount-G. Images were acquired with epifluorescence microscope EVOS FL imaging system (Life Technologies, Cambridge, MA).

Quantification of CNV Size and Vascular Permeability

Images of CNV lesions stained with IB4 were masked and randomized. Lesion exclusion criteria were established before quantification as previously published.^{10,11} All measurements were performed by two different observers in a blinded fashion (L.G.-B., S.D.-T.). Time-course of RUNX1 expression levels was determined by measuring pixel relative fluorescence intensity using ImageJ version 2.0 software (NIH, Bethesda, MD; <http://imagej.nih.gov/ij>). CNV size was measured in a semiautomated manner using the plugin Versatile Wand Tool for ImageJ. Mean CNV area of the lesions of each eye was compared between groups. Vascular permeability was measured by quantifying the leakage area, which corresponds to the difference between the area of the lesion in the late phase and the early phase of the angiogram. Leakage was also measured by grading lesion severity as previously described.¹² Neuroretina and retinal pigment epithelium (RPE)/choroid tissue were collected in lysis buffer and immediately processed for RNA extraction. RNA extraction and transcription into complementary DNA were performed using standard methods for quantitative RT-PCR. Primers for selected genes were purchased from Integrated DNA Technologies (Coralville, IA).

Figure 3 Intravitreal injection of RUNX1 inhibitor reduces CNV area. **A: Top panel:** Quantification of CNV lesion size in the different treatment groups 7 days after laser induction. **Bottom panel:** Representative images of Isolectin B4 labeling of neovascular lesions. **B: Top panel:** Number of mononuclear phagocytes stained with CD11b. **Bottom panel:** Representative images of mononuclear macrophage infiltration in laser CNV lesions. $n = 7$ to 18 mice per group (**A**); $n = 5$ to 8 mice per group (**B**). $*P < 0.05$, $**P < 0.01$. Scale bars: 100 μm . CNV, choroidal neovascularization; MNP, mononuclear phagocytes.

Statistical Analysis

Results are presented as means $\pm$ SEM. Data were assessed with one-way analysis of variance followed by Dunnett's multiple comparisons test. Two-tailed unpaired t -test was used for comparisons between two groups. $P < 0.05$ was considered statistically significant.

Results

RUNX1 Is Expressed in the Main Cell Types Involved in Laser CNV

To investigate the role of RUNX1 in experimental CNV, RUNX1 expression was evaluated by immunostaining of cryosections in response to experimental CNV 7 days post-laser. A wide range of markers of different cell populations known to participate in CNV complex were used.¹³ Positive staining for RUNX1 was detected in all of the cell types studied known to be involved in CNV pathogenesis: endothelial cells (Figure 1A), mononuclear phagocytes (MNP) (ie, macrophages/microglia) (Figure 1B), RPE cells (Figure 1C), and vascular smooth muscle cells/myofibroblasts (Figure 1D). Müller cells, identified by their characteristic shape, also showed RUNX1 expression at the lesion site (Figure 1E). Colocalization was confirmed by confocal microscopy. High expression of RUNX1 was found only at the lesion site, and no RUNX1-positive cells were seen in uninjured retina (Figure 1F).

Time-Course Characterization of RUNX1 Expression Reveals a Peak on Day 3

Fluorescence of choroidal flatmounts stained with RUNX1 was quantified at different time points (Figure 2, A and B), and peak expression was found on day 3 after laser induction. Quantitative RT-PCR analysis revealed increased *RUNX1* mRNA levels in retina (Figure 2C). By contrast, *VEGF* expression remained stable up to day 7 in retinal tissue (Figure 2D). *RUNX1* mRNA expression was significantly up-regulated in RPE/choroid (Figure 2E) at day 3 and day 7. In addition, a significant increase in *VEGF* mRNA levels was observed at day 7 in the RPE/choroid complex (Figure 2F).

Pharmacologic Inhibition of RUNX1 with the Small Molecule Ro5-3335 Reduces CNV Area

To evaluate the preclinical efficacy of RUNX1 modulation in the laser CNV model, an intravitreal injection of either phosphate-buffered saline (vehicle), aflibercept (10 μg), Ro5-3335 (75 $\mu\text{mol/L}$), or a combination of Ro5-3335 + aflibercept (75 $\mu\text{mol/L}$ /10 μg , respectively) was administered immediately after laser photocoagulation. For additional intravitreal Ro5-3335 dose response, see Supplemental Figure S1. Seven days after laser CNV induction, eyes were collected, and the CNV area of flat-mounted choroids stained with IB4 was measured. A single intravitreal injection of Ro5-3335 significantly decreased

Figure 4 Combination of anti-RUNX1 and anti-VEGF is superior at reducing vascular leakage than anti-VEGF monotherapy alone. **A:** Representative images of funduscopy 7 days post-laser and fluorescein angiography early and late phase. **B:** Quantification of CNV leakage area in the different treatment groups. **C:** Fluorescein angiogram severity grading: grade 0 (not leaky), 1 (questionable leakage), 2A (leaky), 2B (clinically significant leakage). **D:** Total number of grade 2B lesions among the different groups. $n = 7$ to 18 mice per group (**B–D**). $*P < 0.05$, $**P < 0.01$. CNV, choroidal neovascularization; VEGF, vascular endothelial growth factor.

lesion size ($12,981.6 \pm 1733.2 \mu\text{m}^2$) compared with vehicle ($27,553.9 \pm 4576.3 \mu\text{m}^2$) (Figure 3A). As expected, a significant reduction in lesion size was also seen with aflibercept ($7979.9 \pm 1154.6 \mu\text{m}^2$), and with the combination of Ro5-3335 + aflibercept ($11,696.3 \pm 2046.1 \mu\text{m}^2$). There were no significant differences in CNV lesion size between groups treated with Ro5-3335 and aflibercept. No significant differences were observed in CD11b-positive (denoting MNPs) cell infiltration quantified in the experimental groups (Figure 3B).

Pharmacologic Inhibition of RUNX1 in Combination with Aflibercept Elicits an Additive Effect in Reducing Vascular Leakage

Vascular permeability was measured by leakage area analysis. Leakage was also measured by grading lesion severity, as previously described.¹² Combination of Ro5-3335 and aflibercept was more effective in reducing vascular leakage area (440.7 ± 302.8 pixels) than aflibercept alone (Figure 4,

A and B). As expected, aflibercept in monotherapy significantly curbed vascular leakage (1510.3 ± 374.0 pixels) compared with vehicle (5023.6 ± 909.5 pixels), whereas Ro5-3335 alone led to a mild reduction that did not reach statistical significance (3542.5 ± 722.4 pixels). Similar results were seen between both qualitative and quantitative analysis, and vascular leakage severity grading indicated highest reduction in leakage in the combination group (Figure 4, C and D).

Discussion

RUNX1 expression was restricted to the CNV area, and positive RUNX1 staining was undetectable elsewhere. By contrast, VEGF is expressed within normal retina even in the absence of active angiogenesis,^{14–16} working as an important neurotrophic factor crucial for survival and normal function of photoreceptors and Müller cells.¹⁷ This suggests that RUNX1 inhibition could have less impact in retinal homeostasis, and additional work investigating its

long-term effects on the retina is warranted. Time-course characterization of *RUNX1* expression revealed peak expression at early time points after laser treatment, whereas *VEGF* expression levels were higher at day 7. Hence, *RUNX1* may play a role in the early pathogenic processes of CNV formation.

The cellular composition of CNV lesions is complex and involves the presence of multiple cell types.^{13,18,19} *RUNX1* expression was found in the primary cell types known to participate in CNV pathogenesis: endothelial cells, MNPs (ie, macrophages/microglia), RPE, Müller cells, and fibroblasts/myofibroblasts. The presence of *RUNX1* in the main cell types in CNV lesions could imply that *RUNX1* participates in multiple processes involved in CNV pathogenesis including aberrant angiogenesis, inflammation, and scar tissue formation.

To evaluate the preclinical efficacy of *RUNX1* inhibition and potential additive or synergistic effects with anti-VEGF therapy, intravitreal injections of vehicle, aflibercept, Ro5-3335, or combination of Ro5-3335 + aflibercept were administered in a laser CNV model. A single intravitreal injection of Ro5-3335 alone significantly decreased CNV lesion size compared with vehicle. Regarding vascular permeability, aflibercept monotherapy significantly decreased leakage, whereas Ro5-3335 alone led to a nonsignificant reduction. Surprisingly, the combination of Ro5-3335 and aflibercept was more effective at reducing CNV leakage when compared with aflibercept alone, suggesting an additive effect of these two drugs. Recently, *TNF α* has been shown to drive *RUNX1* function in endothelial cells by our group, which may further explain the additive effect of *RUNX1* inhibition when combined with anti-VEGF treatment.²⁰ Alternative mechanisms of action in treating CNV may be beneficial for the management of CNV lesions. Based on these data, *RUNX1* inhibition could simultaneously regulate multiple pathologic processes involved in CNV such as aberrant angiogenesis, inflammation, and fibrosis, and therefore, provide a therapeutic advantage over therapies with a limited spectrum of action. This effect on cell types other than endothelial cells may explain the additive effect of *RUNX1* inhibition when combined with anti-VEGF treatment on vascular leakage.

The role of inflammation in neovascular AMD has been previously highlighted,^{21–23} and modulation of inflammatory response has obvious beneficial effects.²⁴ Though the authors did not see a significant difference in the number of MNPs within the CNV lesion, they did observe coexpression of *RUNX1* with MNPs within these cells, suggesting regulation of MNP function. In addition, coexpression of *RUNX1* with MNPs in myofibroblasts suggests another area of further inquiry because subretinal fibrosis is one of the main causes of poor visual outcome in neovascular AMD.²⁵ Additionally, a role for *RUNX1* in mediating epithelial-mesenchymal transition in proliferative vitreoretinopathy, a disease characterized by pathologic fibrosis inside the eye, has been recently shown by our

group.²⁶ Unresolved fluid and progressive fibrosis together with hemorrhage comprise the problem of persistent disease activity despite anti-VEGF therapy, which affects 50% of neovascular AMD patients, and is associated with increased treatment burden and greater visual loss.⁸

In conclusion, these data suggest that *RUNX1* participates in CNV formation through multiple cell types; hence, *RUNX1* inhibition may target not only angiogenesis, but also other processes important in CNV pathogenesis such as inflammation and fibrosis. *RUNX1* inhibition combined with anti-VEGF therapy showed an additive effect in reducing vascular permeability, which leads to intraretinal fluid, the main cause of vision loss in CNV.¹ Therefore, *RUNX1* inhibition alone or in combination with anti-VEGF therapy appears to be a promising therapeutic modality for the treatment of CNV.

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Author Contributions

J.F.A.-V. and L.A.K. initiated this work and supervised all of the aspects of the project; L.G.-B., S.D.-T., J.F.A.-V., and L.A.K. designed the experiments; L.G.-B., S.D.-T., and M.A. conducted and analyzed experiments; M.O.H., D.A., H.A.B.W., and G.Z. provided assistance for the experiments; L.G.-B. and S.D.-T. wrote the manuscript; J.M.R.-M., J.F.A.-V., and L.A.K., revised the manuscript; J.F.A.-V. and L.A.K. are the guarantors of this work and, as such, had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Supplemental Data

Supplemental material for this article can be found at <http://doi.org/10.1016/j.ajpath.2020.12.005>.

References

1. Ritter M, Simader C, Bolz M, Deák GG, Mayr-Sponer U, Sayegh R, Kundi M, Schmidt-Erfurth UM: Intraretinal cysts are the most relevant prognostic biomarker in neovascular age-related macular degeneration independent of the therapeutic strategy. *Br J Ophthalmol* 2014, 98: 1629–1635
2. Schmidt-Erfurth U, Waldstein SM, Deak G-G, Kundi M, Simader C: Pigment epithelial detachment followed by retinal cystoid degeneration leads to vision loss in treatment of neovascular age-related macular degeneration. *Ophthalmology* 2015, 122:822–832
3. Brown DM, Kaiser PK, Michels M, Soubrane G, Heier JS, Kim RY, Sy JP, Schneider S, Group AS: Ranibizumab versus verteporfin for

- neovascular age-related macular degeneration. *N Engl J Med* 2006, 355:1432–1444
4. Rosenfeld PJ, Brown DM, Heier JS, Boyer DS, Kaiser PK, Chung CY, Kim RY, Group MS: Ranibizumab for neovascular age-related macular degeneration. *N Engl J Med* 2006, 355:1419–1431
 5. Rofagha S, Bhisitkul RB, Boyer DS, Sadda SR, Zhang K; SEVEN-UP Study Group: Seven-year outcomes in ranibizumab-treated patients in ANCHOR, MARINA, and HORIZON: a multicenter cohort study (SEVEN-UP). *Ophthalmology* 2013, 120:2292–2299
 6. Comparison of Age-related Macular Degeneration Treatments Trials (CATT) Research Group, Maguire MG, Martin DF, Ying G-S, Jaffe GJ, Daniel E, Grunwald JE, Toth CA, Ferris FL, Fine SL: Five-year outcomes with anti-vascular endothelial growth factor treatment of neovascular age-related macular degeneration: the comparison of age-related macular degeneration treatments trials. *Ophthalmology* 2016, 123:1751–1761
 7. Comparison of Age-related Macular Degeneration Treatments Trials (CATT) Research Group, Martin DF, Maguire MG, Fine SL, Ying G, Jaffe GJ, Grunwald JE, Toth C, Redford M, Ferris FL 3rd: Ranibizumab and bevacizumab for treatment of neovascular age-related macular degeneration: two-year results. *Ophthalmology* 2012, 119:1388–1398
 8. Mettu PS, Allingham MJ, Cousins SW: Incomplete response to Anti-VEGF therapy in neovascular AMD: exploring disease mechanisms and therapeutic opportunities. *Prog Retin Eye Res* 2020, [Epub ahead of print] doi:10.1016/j.preteyeres.2020.100906
 9. Lam JD, Oh DJ, Wong LL, Amarnani D, Park-Windhol C, Sanchez AV, Cardona-Velez J, McGuone D, Stemmer-Rachamimov AO, Elliott D, Bielenberg DR, van Zyl T, Shen L, Gai X, D'Amore PA, Kim LA, Arboleda-Velasquez JF: Identification of RUNX1 as a mediator of aberrant retinal angiogenesis. *Diabetes* 2017, 66:1950–1956
 10. Gong Y, Li J, Sun Y, Fu Z, Liu C-H, Evans L, Tian K, Saba N, Fredrick T, Morss P, Chen J, Smith LEH: Optimization of an image-guided laser-induced choroidal neovascularization model in mice. *PLoS One* 2015, 10:e0132643
 11. Poor SH, Qiu Y, Fassbender ES, Shen S, Woolfenden A, Delpero A, Kim Y, Buchanan N, Gebuhr TC, Hanks SM, Meredith EL, Jaffee BD, Dryja TP: Reliability of the mouse model of choroidal neovascularization induced by laser photocoagulation. *Invest Ophthalmol Vis Sci* 2014, 55:6525–6534
 12. Krzystolik MG, Afshari MA, Adamis AP, Gaudreault J, Gragoudas ES, Michaud NA, Li W, Connolly E, O'Neill CA, Miller JW: Prevention of experimental choroidal neovascularization with intravitreal anti-vascular endothelial growth factor antibody fragment. *Arch Ophthalmol* 2002, 120:338
 13. Espinosa-Heidmann DG, Reinoso MA, Pina Y, Csaky KG, Caicedo A, Cousins SW: Quantitative enumeration of vascular smooth muscle cells and endothelial cells derived from bone marrow precursors in experimental choroidal neovascularization. *Exp Eye Res* 2005, 80:369–378
 14. Saint-Geniez M, Maldonado AE, D'Amore PA: VEGF expression and receptor activation in the choroid during development and in the adult. *Invest Ophthalmol Vis Sci* 2006, 47:3135
 15. Blaauwgeers HGT, Holtkamp GM, Rutten H, Witmer AN, Koolwijk P, Partanen TA, Alitalo K, Kroon ME, Kijlstra A, van Hinsbergh VWM, Schlingemann RO: Polarized vascular endothelial growth factor secretion by human retinal pigment epithelium and localization of vascular endothelial growth factor receptors on the inner choriocapillaris. *Am J Pathol* 1999, 155:421–428
 16. Pierce EA, Avery RL, Foley ED, Aiello LP, Smith LE: Vascular endothelial growth factor/vascular permeability factor expression in a mouse model of retinal neovascularization. *Proc Natl Acad Sci U S A* 1995, 92:905–909
 17. Saint-Geniez M, Kurihara T, Sekiyama E, Maldonado AE, D'Amore PA: An essential role for RPE-derived soluble VEGF in the maintenance of the choriocapillaris. *Proc Natl Acad Sci U S A* 2009, 106:18751–18756
 18. Lopez PF, Grossniklaus HE, Lambert HM, Aaberg TM, Capone A, Sternberg P, L'Hernault N: Pathologic features of surgically excised subretinal neovascular membranes in age-related macular degeneration. *Am J Ophthalmol* 1991, 112:647–656
 19. Giani A, Thanos A, Roh MI, Connolly E, Trichonas G, Kim I, Gragoudas E, Vavvas D, Miller JW: In vivo evaluation of laser-induced choroidal neovascularization using spectral-domain optical coherence tomography. *Invest Ophthalmol Vis Sci* 2011, 52:3880–3887
 20. Whitmore HAB, Amarnani D, O'Hare M, Delgado-Tirado S, Gonzalez-Buendia L, An M, Pedron J, Bushweller JH, Arboleda-Velasquez JF, Kim LA: TNF- α signaling regulates RUNX1 function in endothelial cells. *FASEB J* 2020, [Epub ahead of print] doi:10.1096/fj.202001668R
 21. Ambati J, Atkinson JP, Gelfand BD: Immunology of age-related macular degeneration. *Nat Rev Immunol* 2013, 13:438–451
 22. Knickelbein JE, Chan C-C, Sen HN, Ferris FL, Nussenblatt RB: Inflammatory mechanisms of age-related macular degeneration. *Int Ophthalmol Clin* 2015, 55:63–78
 23. Feng L, Ju M, Lee KYV, Mackey A, Evangelista M, Iwata D, Adamson P, Lashkari K, Foxton R, Shima D, Ng YS: A proinflammatory function of toll-like receptor 2 in the retinal pigment epithelium as a novel target for reducing choroidal neovascularization in age-related macular degeneration. *Am J Pathol* 2017, 187:2208–2221
 24. Wang Y, Wang VM, Chan C-C: The role of anti-inflammatory agents in age-related macular degeneration (AMD) treatment. *Eye* 2011, 25:127–139
 25. Okeagu CU, Agrón E, Vitale S, Domalpally A, Chew EY, Keenan TDL; AREDs2 Research Group: Principal cause of poor visual acuity following neovascular age-related macular degeneration: AREDs2 report number 23. *Ophthalmol Retina* 2021, 5:23–31
 26. Delgado-Tirado S, Amarnani D, Zhao G, Rossin EJ, Elliott D, Miller JB, Greene WA, Ramos L, Arevalo-Alquichire S, Leyton-Cifuentes D, Gonzalez-Buendia L, Isaacs-Bernal D, Whitmore HAB, Chmielewska N, Duffy BV, Kim E, Wang H-C, Ruiz-Moreno JM, Kim LA, Arboleda-Velasquez JF: Topical delivery of a small molecule RUNX1 transcription factor inhibitor for the treatment of proliferative vitreoretinopathy. *Sci Rep* 2020, 10:20554