Research article

Multimodal imaging to detect in vivo responses to aflibercept therapy in a mouse model of type 3 neovascularization

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Short title: Type 3 neovascularization mouse model

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ABSTRACT

Purpose: To characterize the response to aflibercept in a mouse model of type 3 neovascularization, the neoretinal vascularization (NRV) 2 mouse line.

Methods: Twelve NRV2 mice were assigned to one of the following groups: (i) six mice were injected with aflibercept 3µg/g at postnatal day 15 ("aflibercept" group), and (ii) the remaining six mice did not receive any treatment ("placebo" group). Mice were examined at postnatal day 30 (p30) and 44 (p44).

Results: The NRV mice's retinas were characterized by regions of depigmentation that were topographically associated with hyperfluorescent lesions seen on fluorescein angiography (FA) images. On optical coherence tomography (OCT) images, intraretinal neovascularizations were visualized as hyperreflective lesions mainly localized within the outer plexiform and outer nuclear layers. The average number of intraretinal neovascular lesions visualized on FA at P30 was 5.0±2.2 in the "aflibercept" and 20.7±2.4 in the "placebo" groups ($p<0.0001$). At P44, the average number of intraretinal lesions was still lower in the "aflibercept" group, although this difference was not statistically significant ($p=0.088$).

Conclusion: Aflibercept therapy was effective in inhibiting the pathologic angiogenesis in the NRV2 mouse model. However, the successive treatment washout resulted in an increase in lesions' number.

INTRODUCTION

Age-related macular degeneration (AMD) is the leading cause of central vision loss among older individuals in developed countries and severe vision loss is often the result of macular neovascularization (NV) development.(1) In neovascular AMD, exudation may occur from pathologic type 1 (sub-retinal pigment epithelium - RPE), type 2 (sub-retinal), or type 3 (intra-retinal) NV. Type 3 NV represents a distinct form of intraretinal neovascularization that was first identified by Hartnett et al(2) in 1992. This entity was initially termed retinal angiomatous proliferation (RAP)(3) and successively renamed type 3 NV.(4–6)

In the last decades, several animal models have been conceived in order to better characterize ocular vascular disorders.(7) Importantly, mutations or dysregulation of distinct genes resulting in type 3 NV development were applied to transgenic mouse models.(8–13) Among the mouse models of type 3 NV, the neoretinal vascularization (NRV) 2 mouse line, also called JR5558 mice, is a recently discovered spontaneous model which exhibits presence of intraretinal neovascularizations.(13) In details, this model was demonstrated to be characterized by multiple regions of retinal depigmentation and spontaneous development of intraretinal neovascularizations arising from the retinal deep vascular complex (DVC) and extending toward the RPE. The latter attributes were similar to those displayed in type 3 NV appearing in human eyes, as *in vivo* imaging technologies demonstrated that type 3 NVs emerge from the DVC and form a clear tuft-shaped high-flow network in the outer retinal layers and finally abut in the sub-RPE space.(14–20) Of note, although the exact mechanisms leading to intraretinal neovascularization in NRV2 mouse model

are not well known, this mutant mouse model seems to have a recessive mode of inheritance.

The introduction of anti-vascular endothelial growth factor (VEGF) drugs has radically changed the treatment of exudative AMD.(21) Aflibercept (Eylea; Regeneron Pharmaceuticals, Tarrytown, NY and Bayer HealthCare Pharmaceuticals, Berlin, Germany) is a recombinant protein composed of the binding domains of two human VEGF receptors fused with the Fc region of human immunoglobulin gamma 1 (IgG1). Its use for exudative neovascular AMD was approved by the U.S. Food and Drug Administration in 2011.

Work in preclinical models has paved the way for evaluation of the efficacy and development of antiangiogenic therapies, including anti-VEGF therapies for the treatment of exudative neovascular AMD.(22–24) However, detailed description of the retinal effects of aflibercept therapy on preclinical models of type 3 NV has not been performed. Therefore, the aim of this study was to characterize progressive retinal changes occurring after aflibercept therapy using in vivo longitudinal imaging in NRV2 mouse model.

METHODS

Animals

This experimental study was approved by the Institutional Animal Care and Animals were Use Committee (IACUC) of the San Raffaele Scientific Institute in

Milan and all animal procedures adhered to the National Legislation (D.L. 116/1992) and the European Directive (2010/63/EU). Twelve NRV2 (or JR5558) mice were obtained from The Jackson Laboratory (Bar Harbor, ME).

At postnatal day 15, the experimental mice were randomly assigned to one of the following groups: (i) six mice were injected intraperitoneally with aflibercept 3μg/g ("aflibercept" group), and (ii) the remaining six mice did not receive any treatment ("placebo" group).

In vivo retinal analysis

In vivo imaging evaluations were performed in the right eye at postnatal days (P)30 and 44 (15 and 29 days after aflibercept injection in the treatment group, respectively), as previously described.(13) In brief, before the imaging session, the mice's pupils were dilated with 0.5% tropicamide (Visumidriatic, Tubilux Pharma, Pomezia, Italy). For each mouse, the right eye underwent the imaging session. Color fundus photography, fluorescein angiography (FA) and optical coherence tomography (OCT) images were taken using the Phoenix MICRON IV retinal imaging microscope device (Phoenix Research Laboratories, Pleasanton, CA). FA images were taken at early (1 minute after fluorescein injection, middle (3–5 minutes after fluorescein injection), and late (10 minutes after fluorescein injection) phases. Importantly, OCT images were taken under the FA guidance in order to assess structural changes topographically co-localizing with hyperfluorescent regions on FA.

Grading and statistical analysis

The FA and OCT findings were carefully examined by an experienced grader. In details, the grading analysis was aimed at assessing: (i) the number of hyperfluorescent lesions seen on FA images; and (ii) the structural features of these lesions on OCT images. The analysis included qualitative descriptions of the imaging characteristics. In addition, the average number of hyperfluorescent lesions was compared between the two groups by conducting a Student T-test for independent variables. To detect departures from normal distribution, a Shapiro-Wilk's test was performed. Differences were considered as statistically significant with a p value of 0.05.

RESULTS

Qualitative analysis

At both postnatal days 30 and 44, fundus assessment displayed the presence of diffuse areas of depigmentation. These regions of depigmentation were topographically associated with hyperfluorescent lesions seen on FA images (Figures 1-3). Furthermore, these lesions were characterized by late leakage indicating the presence of active neovascular lesions.

On OCT images, these intraretinal neovascularizations were visualized as hyperreflective lesions within the outer plexiform (OPL) and outer nuclear (ONL) layers. Moreover, some of these lesions reached either the ellipsoid zone (EZ) or RPE layers, this probably reflecting distinct temporal stages in the intraretinal neovascularization development, characterized by extension toward the RPE and abutting in the sub-RPE space (Figures 1-3).

Qualitatively, the "aflibercept" group demonstrated a lower number of these

lesions at P30 (15 days after aflibercept injection) compared with the “placebo” group, as assessed using the three imaging modalities. At P44 (29 days after aflibercept injection), the number of lesions seemed to be increased within the “aflibercept” group, although the density of these lesions was still reduced in comparison with the “placebo” group.

Quantitative analysis

The qualitative grading was confirmed by the quantitative analysis. In details, the average number of intraretinal lesions visualized on FA imaging at P30 was 5.0±2.2 in the “aflibercept” group and 20.7±2.4 in the “placebo” group ($p<0.0001$). At P44, the average number of intraretinal lesions was still lower in the “aflibercept” group, although this difference was not statistically significant (13.2±4.3 in the “aflibercept” group and 17.7±3.1 in the “placebo” group, $p=0.088$) (Figure 4).

DISCUSSION

In this preclinical study employing *in vivo* retinal imaging we investigated ultrastructural changes occurring after aflibercept therapy in an established mouse model developing intraretinal neovascular lesions. Overall, we confirmed that NRV2 mice spontaneously develop retinal depigmentation and vascular leakage in the retina within the early postnatal days. More importantly, we demonstrated that aflibercept administration significantly reduces the number of type 3 lesions in NRV2 mice undergoing treatment, in comparison with mice untreated.

Aflibercept was conceived to treat both malignant solid tumors and ocular vascular disorders. Orthotopic mouse models were helpful to demonstrate that aflibercept is effective in decreasing tumor growth in different malignant cancers.(25) Notably, ocular preclinical studies were aimed at displaying that aflibercept has *in vivo* activity toward various murine models of NV. In details, aflibercept was displayed to prevent the development of NV in mice treated with laser photocoagulation, in VEGF-secreting transgenic mouse models, and in mice receiving exogenous VEGF.(26) The latter beneficial responses were demonstrated to be associated with reductions in both endothelial nitric oxide synthetase synthesis and intercellular adhesion molecule-1 within the NV.(27) Furthermore, in mice developing matrigel-induced NV, aflibercept injections at 2 and 6 days precluded the NV development, while injections at 10 days reduced the inflammatory response.(28) These favorable evidences from preclinical studies supported the development of large clinical trials testing aflibercept for neovascular exudative AMD in humans. (29)

The NRV2 mouse model was first characterized by Hasegawa et al(13) who employed *in vivo* and *ex vivo* approaches to describe intraretinal lesions developing in this preclinical model. Our results confirmed that NRV2 mice spontaneously develop retinal depigmentation and vascular leakage within the early postnatal days. Furthermore, OCT analysis confirmed the presence of hyperreflective structures in the outer retinal layers. Although we were not able to provide optical coherence tomography (OCTA) images, our findings seem to be consistent with the presence of intraretinal neovessels originating from the

DVC. Importantly, in agreement with histopathological studies that have failed to identify any choroidal connection associated with intraretinal type 3 NV in humans,(30,31) also the histological analysis provided by Hasegawa and colleagues(13) did not display connections between retinal neovessels and choroidal vessels.

Several authors supposed that the development of type 3 NV arising from the DVC in humans may be driven by a disproportion of VEGF and other angiogenic molecules emerging from the apical side of the RPE cells.(32,33) This hypothesis was further corroborated by the evidence that the aqueous humor levels of VEGF are more elevated in eyes with type 3 NV versus eyes with type 1 or type 2 NV.(34) Similarly, Hasegawa et al(13) measured the VEGF levels in the retina of NRV2 mice and demonstrated that this molecule was significantly increased in comparison with control mice, this suggesting that the development of intraretinal neovascularizations in this mouse model may be in part associated with VEGF overexpression. However, the latter study was not able to elucidate whether the VEGF overexpression reflects a shared underlying mechanism or it represents a causative mechanism resulting in the development of intraretinal neovascularizations.

We add to the literature by reporting the effects of aflibercept in a preclinical model of type 3 NV. We demonstrated that NRV2 mice undergoing aflibercept treatment have a significant reduction in number of intraretinal neovascularizations. Thus, exogenous administration of aflibercept induces, in principle, a protective effect in NRV2 mice, as observed in our model. However,

the successive treatment washout results in an increase in lesions' number. Our results seem to corroborate the hypothesis that VEGF overexpression and development of intraretinal neovascularizations represent tributary mechanisms. These findings further confirm that the origin and progression of intraretinal angiogenesis in the NRV2 mouse model is strictly VEGF-dependent and it excellently resembles human type 3 NV.

A main limitation is that aflibercept therapy was injected intraperitoneally rather than via intravitreal injection. However, different previous studies employed this type of instillation which was demonstrated to cause significant changes in the retina.(35) Furthermore, our sample size was limited to 12 mice. Future studies with larger sample sizes will be needed to confirm our findings. A final limitation was that we did not perform imaging before treatment with anti-VEGF. However, the aim of our study was to detect differences between mice treated and not treated with anti-VEGF.

In conclusion, this study employed *in vivo* retinal imaging to investigate ultrastructural alterations occurring in an established mouse model developing intraretinal neovascular lesions. Furthermore, we investigated the effects of aflibercept therapy in these mice. Our findings confirmed that the intraretinal neovessels developing in the NRV2 mouse strongly resemble human type 3 NVs and are responsive to aflibercept treatment. Therefore, this mouse model was further proved to be the useful in evaluating characteristics of type 3 NV and to study novel therapeutic approaches for this sub-type of NV.

Statement of Ethics

This experimental study was approved by the Institutional Animal Care and Animals were Use Committee (IACUC) of the San Raffaele Scientific Institute in Milan and all animal procedures adhered to the National Legislation (D.L. 116/1992) and the European Directive (2010/63/EU).

Conflict of Interest Statement

The authors have the following disclosures: Francesco Bandello is a consultant for: Alcon (Fort Worth,Texas,USA), Alimera Sciences (Alpharetta, Georgia, USA), Allergan Inc (Irvine, California,USA), Farmila-Thea (Clermont-Ferrand, France), Bayer Shering-Pharma (Berlin, Germany), Bausch And Lomb (Rochester, New York, USA), Genentech (San Francisco, California, USA), Hoffmann-La-Roche (Basel, Switzerland), Novagali Pharma (Évry, France), Novartis (Basel, Switzerland), Sanofi-Aventis (Paris, France), Thrombogenics (Heverlee,Belgium), Zeiss (Dublin, USA). Giuseppe Querques is a consultant for: Alimera Sciences (Alpharetta, Georgia, USA), Allergan Inc (Irvine, California, USA), Amgen (Thousand Oaks ,USA), Bayer Shering-Pharma (Berlin, Germany), Heidelberg (Germany), KBH (Chengdu; China), LEH Pharma (London, UK), Lumithera (Poulsbo; USA), Novartis (Basel, Switzerland), Sandoz (Berlin, Germany), Sifi (Catania, Italy), Sooft-Fidea (Abano, Italy), Zeiss (Dublin, USA). The other authors have no disclosures.

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Author Contributions

EB: Data analysis and statistics. Drafting, revision and final approval of manuscript.

GZ: Concept and study design. Data interpretation. Data analysis and statistics. Revision and final approval of manuscript.

SM: Concept and study design. Data interpretation. Data analysis and statistics. Revision and final approval of manuscript.

RS: Revision and final approval of manuscript.

LQ: Revision and final approval of manuscript.

IZ: Revision and final approval of manuscript.

FB: Concept and study design. Revision and final approval of manuscript.

GQ: Concept and study design. Data interpretation. Data analysis and statistics. Revision and final approval of manuscript.

REFERENCES

1. Jager RD, Mieler WF, Miller JW. Age-Related Macular Degeneration. N Engl J Med [Internet]. 2008 [cited 2018 Feb 1]; 358: 2606–17. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18550876

2. Hartnett ME, Weiter JJ, Garsd A, Jalkh AE. Classification of retinal pigment epithelial detachments associated with drusen. Graefe's Arch Clin Exp Ophthalmol [Internet]. 1992 [cited 2017 Nov 7]; 230: 11–9. Available from: https://www.ncbi.nlm.nih.gov/m/pubmed/1547961/?i=3&from=Classification of retinal pigment epithelial detachments associated with drusen

3. Yannuzzi LA, Negrão S. RETINAL ANGIOMATOUS PROLIFERATION IN AGE–RELATED MACULAR DEGENERATION. RETINA. 2001; 21: 416–34.

4. Yannuzzi LA, Freund KB, Takahashi BS. Review of retinal angiomatous proliferation or type 3 neovascularization. Retina [Internet]. 2008; 28: 375–84. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18327130

5. Freund KB, Ho I-V, Barbazetto I a, et al. Type 3 neovascularization: the expanded spectrum of retinal angiomatous proliferation. Retina. 2008; 28: 201–11.

6. Freund KB, Zweifek S, Engelbert M. Editorial Do We Need a New Classification for Choroidal Neovascularization in Age-Related Macular. Retina. 2010; 30: 1333–49.

7. Liu CH, Wang Z, Sun Y, Chen J. Animal models of ocular angiogenesis: From development to pathologies. FASEB J. 2017;

8. Heckenlively JR, Hawes NL, Friedlander M, et al. Mouse model of subretinal neovascularization with choroidal anastomosis. Retina. 2003;

9. Li C, Huang Z, Kingsley R, et al. Biochemical alterations in the retinas of very low-density lipoprotein receptor knockout mice: An animal model of retinal angiomatous proliferation. Arch Ophthalmol. 2007;

10. Okamoto N, Tobe T, Hackett SF, et al. Transgenic mice with increased expression of vascular endothelial growth factor in the retina: A new model of intraretinal and subretinal neovascularization. Am J Pathol. 1997;

11. Tobe T, Okamoto N, Vinores MA, et al. Evolution of neovascularization in mice with overexpression of vascular endothelial growth factor in photoreceptors. Investig Ophthalmol Vis Sci. 1998;

12. Hu W, Jiang A, Liang J, et al. Expression of VLDLR in the retina and evolution of subretinal neovascularization in the knockout mouse model's retinal angiomatous proliferation. Investig Ophthalmol Vis Sci. 2008;

13. Hasegawa E, Sweigard H, Husain D, et al. Characterization of a spontaneous retinal neovascular mouse model. PLoS One. 2014;

14. Querques G, Querques L, Forte R, Massamba N, Blanco R, Souied EH. Precursors of type 3 neovascularization: A multimodal imaging analysis. Retina [Internet]. 2013; 33: 1241–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23508076

15. Querques G, Souied EH, Freund KB. MULTIMODAL IMAGING OF EARLY STAGE 1 TYPE 3 NEOVASCULARIZATION WITH SIMULTANEOUS EYE-TRACKED SPECTRAL-DOMAIN OPTICAL

COHERENCE TOMOGRAPHY AND HIGH-SPEED REAL-TIME ANGIOGRAPHY. Retina [Internet]. 2013 [cited 2017 Nov 7]; 33: 1881–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23644560

16. Nagiel A, Sarraf D, Sadda SR, et al. Type 3 neovascularization: Evolution, association with pigment epithelial detachment, and treatment response as revealed by spectral domain optical coherence tomography. Retina. 2015; 35: 638–47.

17. Su D, Lin S, Phasukkijwatana N, et al. An Updated Staging System of Type 3 Neovascularization Using Spectral Domain Optical Coherence Tomography. Retina. 2016; : 40–9.

18. Kuehlewein L, Dansingani KK, de Carlo TE, et al. Optical Coherence Tomography Angiography of Type 3 Neovascularization Secondary To Age-Related Macular Degeneration. Retina [Internet]. 2015; 35: 2229–35. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26502007%5Cnhttp://content.wkhealth.com/linkback/openurl?sid=WKPTLP:landingpage&an=00006982-201511000-00009

19. Phasukkijwatana N, Tan ACS, Chen X, Freund KB, Sarraf D. Optical coherence tomography angiography of type 3 neovascularisation in age-related macular degeneration after antiangiogenic therapy. Br J Ophthalmol. 2016;

20. Borrelli E, Sacconi R, Klose G, de Sisternes L, Bandello F, Querques G. Rotational Three-dimensional OCTA: a Notable New Imaging Tool to Characterize Type 3 Macular Neovascularization. Sci Rep. 2019;

21. Dorrell M, Uusitalo-Jarvinen H, Aguilar E, Friedlander M. Ocular Neovascularization: Basic Mechanisms and Therapeutic Advances. Surv Ophthalmol. 2007;

22. Miao HQ, Hu K, Jimenez X, et al. Potent neutralization of VEGF biological activities with a fully human antibody Fab fragment directed against VEGF receptor 2. Biochem Biophys Res Commun. 2006;

23. Browning DJ, Kaiser PK, Rosenfeld PJ, Stewart MW. Aflibercept for age-related macular degeneration: A game-changer or quiet addition? Am. J. Ophthalmol. 2012;

24. Maestroni S, Maestroni A, Ceglia S, et al. Effect of chromogranin A-derived vasostatin-1 on laser-induced choroidal neovascularization in the mouse. Acta Ophthalmol. 2015;

25. Stewart MW. Aflibercept (VEGF-TRAP): The next anti-VEGF drug. Inflamm Allergy - Drug Targets. 2011;

26. Saishin Y, Saishin Y, Takahashi K, et al. VEGF-TRAPR1R2 suppresses choroidal neovascularization and VEGF-induced breakdown of the blood-retinal barrier. J Cell Physiol. 2003;

27. Joussen AM, Poulaki V, Qin W, et al. Retinal vascular endothelial growth factor induces intercellular adhesion molecule-1 and endothelial nitric oxide synthase expression and initiates early diabetic retinal leukocyte adhesion in vivo. Am J Pathol. 2002;

28. Cao J, Zhao L, Li Y, et al. A subretinal matrigel rat choroidal neovascularization (CNV) model and inhibition of CNV and associated Inflammation and fibrosis by VEGF Trap. Investig Ophthalmol Vis Sci.

2010;

29. Heier JS, Brown DM, Chong V, et al. Intravitreal aflibercept (VEGF trap-eye) in wet age-related macular degeneration. Ophthalmology. 2012;

30. Shimada H, Kawamura A, Mori R, Yuzawa M. Clinicopathological findings of retinal angiomatous proliferation. Graefe's Arch Clin Exp Ophthalmol [Internet]. 2007 [cited 2018 Mar 11]; 245: 295–300. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16738855

31. Monson DM, Smith JR, Klein ML, Wilson DJ. Clinicopathologic Correlation of Retinal Angiomatous Proliferation. Arch Ophthalmol [Internet]. 2008 [cited 2018 Mar 11]; 126: 1664. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19064845

32. Holz FG, Pauleikhoff D, Klein R, Bird AC. Pathogenesis of lesions in late age-related macular disease. Am. J. Ophthalmol. 2004; 137: 504–10.

33. Spaide RF. Fundus autofluorescence and age-related macular degeneration. Ophthalmology [Internet]. 2003 [cited 2018 Mar 11]; 110: 392–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12578786

34. Dell'Omo R, Cassetta M, Dell'Omo E, et al. Aqueous humor levels of vascular endothelial growth factor before and after intravitreal bevacizumab in type 3 versus type 1 and 2 neovascularization. A prospective, case-control study. Am J Ophthalmol. 2012; 153.

35. Dose-dependency of intraperitoneally-injected aflibercept in the mouse choroidal neovascularization model. Acta Ophthalmol. 2018;

FIGURE LEGENDS

Figure 1. Images from a representative normal mouse.

(**A**) The fundus image shows a normal pigmentation of the retina. (**B**) The fluorescein angiography image displays the absence of hyperfluorescent lesions and leakage. (**C**) The optical coherence tomography (OCT) image demonstrates a normal appearance of the inner and outer retinal layers.

Figure 2. Images from a representative NRV2 mouse not undergoing treatment.

(**A**) The fundus images taken at P30 show areas whit depigmentation which co-localize with hyperfluorescent regions with vascular leakage on late phase fluorescein angiography (FA) (**B**). (**C**) The optical coherence tomography (OCT) image demonstrates presence of hyperreflective lesions that are mainly localized in the in the outer nuclear (ONL) and outer plexiform (OPL) layers of the retina. The same imaging modalities were obtained at P44 (**D,E,F**) and demonstrated an overall stability of the vascular lesions. Please note that red arrowheads are pointed to some regions with retinal disturbances.

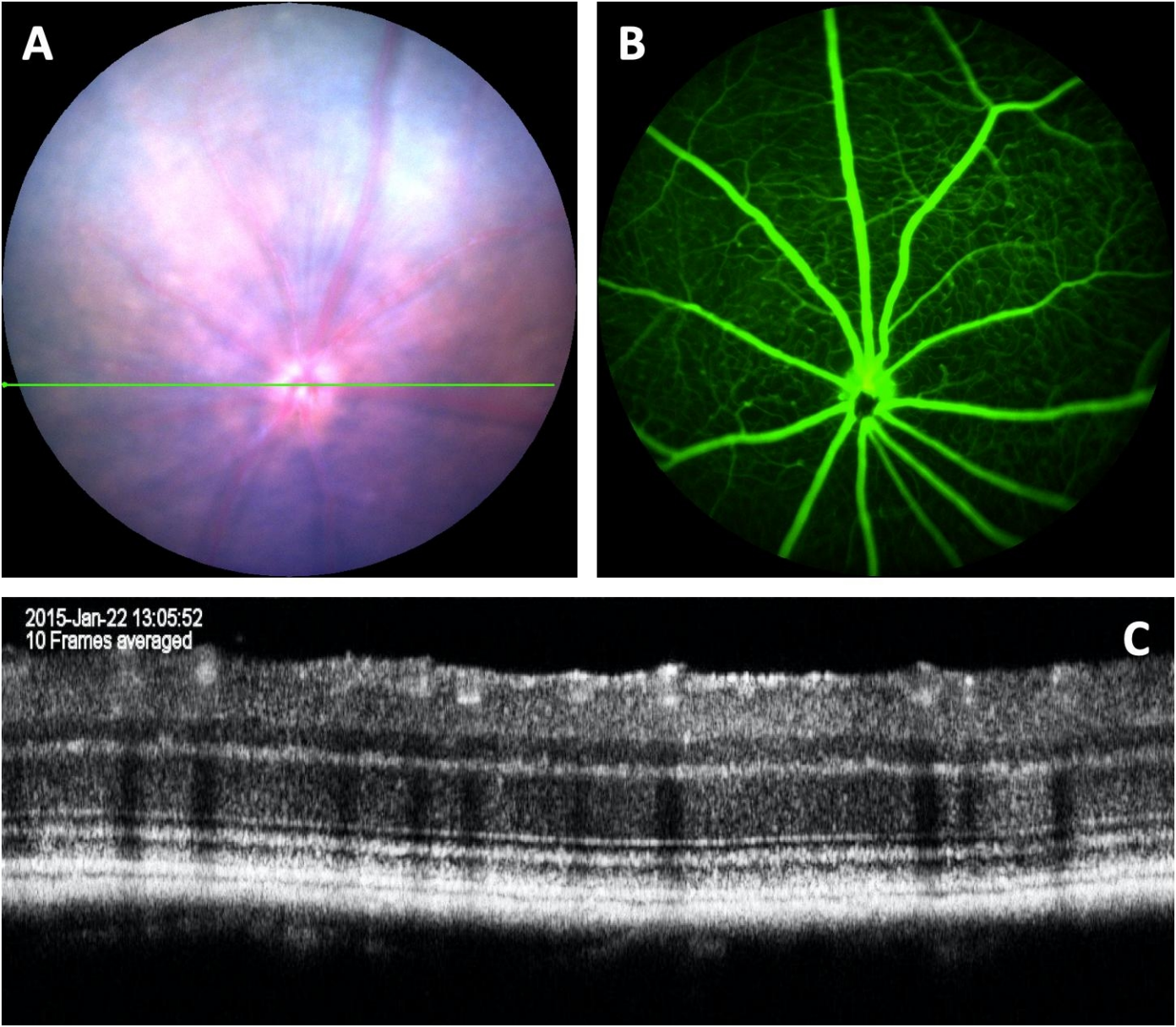
Figure 3. Images from a representative NRV2 mouse undergoing aflibercept treatment.

(**A**) The fundus images taken at P30 (15 days after aflibercept treatment) show a few areas whit slight depigmentation co-localizing with hyperfluorescent and leaking vascular lesions on late phase fluorescein angiography (FA) (**B**). (**C**) The optical coherence tomography (OCT) image demonstrates presence of hyperreflective lesions that are mainly localized in the in the outer nuclear layer

(ONL). The same imaging modalities performed at P44 (29 days after aflibercept treatment **- D,E,F**) displayed an increased number of vascular lesions because of treatment washout. Please note that colored arrowheads are pointed to some regions with retinal disturbances and different colors indicate topographical correspondences between different imaging modalities.

Figure 4. Scatter plot showing the average number of neovascular lesions in untreated ("sham") and treated ("aflibercept") NRV2 mice at P30 and P44.

Each black dot shows the average number of lesions in an analyzed mouse. Black horizontal lines indicate the mean value of each group.


A
B
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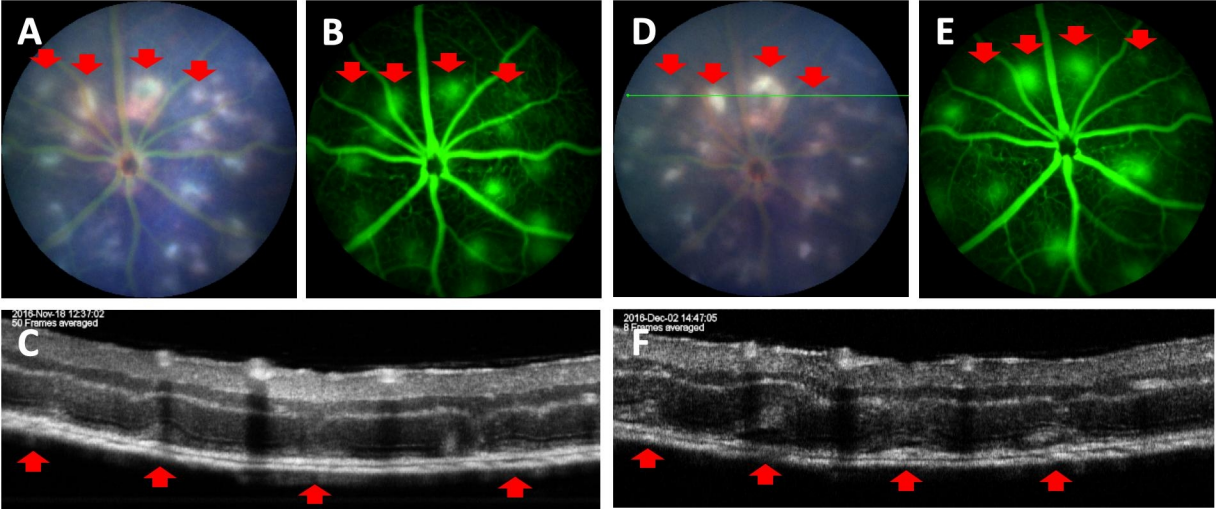
10 Frames averaged
C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B
D
E
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C
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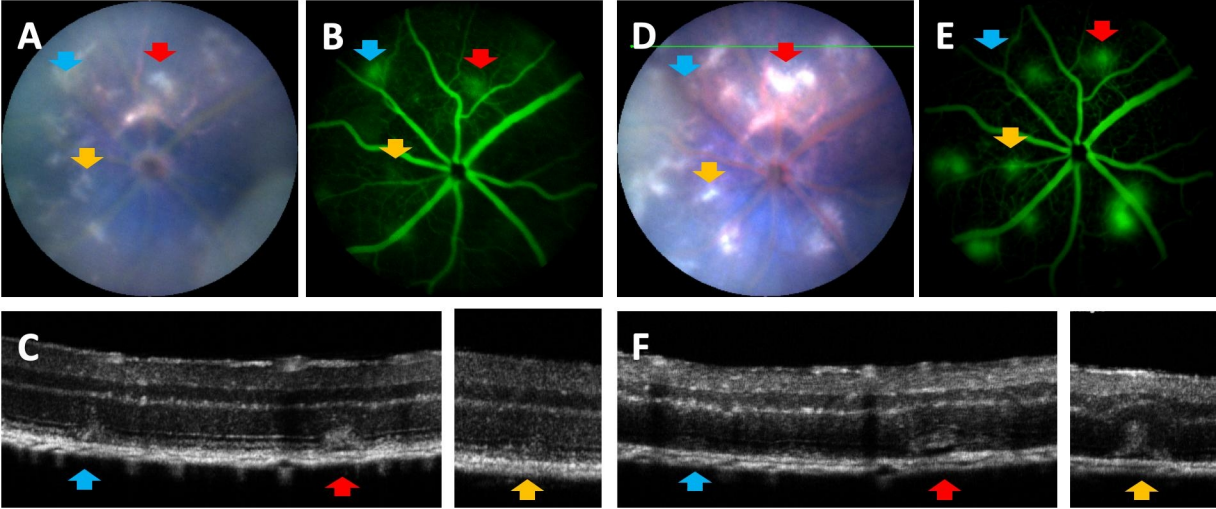
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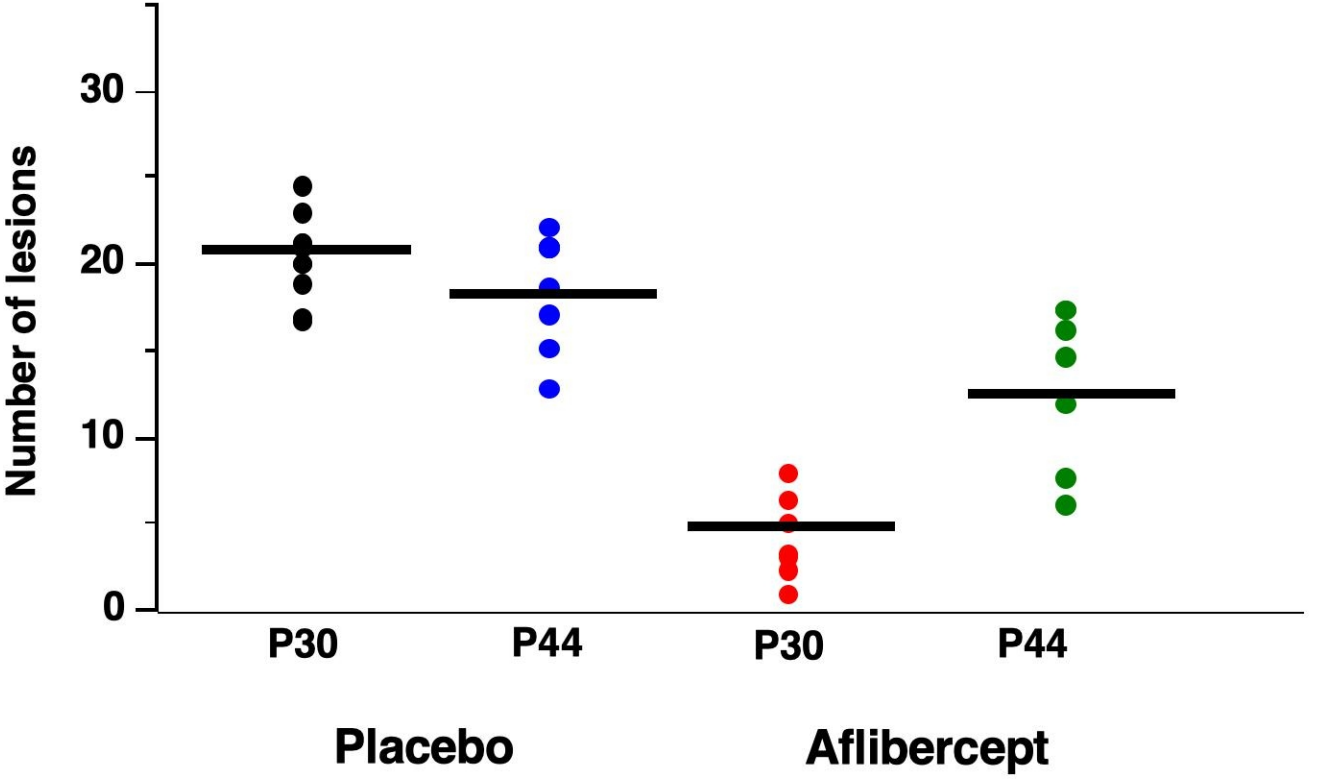
Number of lesions
30
20
10
0
P30
P44
P30
P44
Placebo
Aflibercept