CURRENT EYE RESEARCH
2025, VOL. 50, NO. 4, 410–419
https://doi.org/10.1080/02713683.2024.2441245

Taylor & Francis
Taylor & Francis Group

Brolucizumab and Platelet Activation and Reactivity

B. Sobolewska[a], S. Poeschel[b], H. Kalbacher[c], K. Bieber[b], A. M. Paczulla Stanger[b], Konstantinos Stellos[d,e,f,g,h]* and F. Ziemssen[a]

[a]Centre for Ophthalmology, Eberhard-Karls University, Tübingen, Germany; [b]Department of Internal Medicine II, Core Facility Flow Cytometry of the Medical Faculty Tübingen, University of Tübingen, Tübingen, Germany; [c]Interfaculty Institute of Biochemistry, Eberhard-Karls University of Tuebingen, Tübingen, Germany; [d]Department of Cardiovascular Research, Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany; [e]Department of Cardiology, Preventive Cardiology Clinic, University Hospital Mannheim, Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany; [f]German Centre for Cardiovascular Research (DZHK), Partner Site Heidelberg/Mannheim, Mannheim, Germany; [g]Department of Medicine, University Medical Centre Mannheim, Heidelberg University, Mannheim, Germany; [h]Biosciences Institute, Vascular Biology and Medicine Theme, Faculty of Medical Sciences, Newcastle University, Newcastle Upon Tyne, UK

ABSTRACT

Purpose: This study explores the potential interaction of brolucizumab with platelets and its effects on platelet activation and reactivity, crucial in retinal vasculitis and retinal vascular occlusion. Safety concerns remain of interest, although brolucizumab showed superior retinal efficacy and reduced injection frequency compared to other licensed anti-VEGF agents.

Methods: Resting and activated platelets of healthy volunteers were pretreated with brolucizumab at the following concentrations 0.6 µg/mL, 3 µg/mL, 6 µg/mL, 300 µg/mL, and 3000 µ/mL or its solvent or PBS. The surface expression of platelet activation markers GPIIb/IIIa and P-selectin was determined by multispectral imaging flow cytometry, which combines flow cytometry and fluorescence microscopy. Two different methods were used to examine the interaction of brolucizumab with platelets: 1) A cross-pretreatment experiment was performed with FITC-labeled brolucizumab and bevacizumab; 2) Resting and activated platelets were pretreated with brolucizumab or its solvent or PBS, followed by anti-brolucizumab antibody generated by rabbit immunization.

Results: Brolucizumab did not significantly affect platelet activation compared to solvent or PBS, across a range of concentrations. No significant upregulation of CD62P and no activation of the fibrinogen receptor (GPIIb/IIa) were observed in resting and TRAP-activated platelets. After pretreatment with PBS, the level of brolucizumab-FITC was significantly lower in comparison to bevacizumab-FITC (normalized MFI = 3.32, CI = 3.16–3.48 vs. normalized MFI = 7.19, CI = 7.04–7.35; $p < 0.001$). Both brolucizumab- and bevacizumab-FITC were downregulated after pretreatment with brolucizumab or bevacizumab compared to pretreatment with PBS. Antibodies against brolucizumab did not show any significant difference between pretreatment with brolucizumab and its solvent in resting and TRAP-activated platelets.

Conclusion: Brolucizumab does not appear to directly affect platelet activation or reactivity to thrombin receptor agonists. No platelet interaction was observed after increasing brolucizumab concentrations or anti-brolucizumab antibodies in resting and activated platelets. However, brolucizumab might be taken up in platelets.

ARTICLE HISTORY
Received 12 May 2024
Accepted 4 December 2024

KEYWORDS
Brolucizumab; anti-drug antibodies; adverse events; platelets; platelet activation

Introduction

Brolucizumab (Brolucizumab®) is the smallest anti-VEGF agent approved for the treatment of neovascular age-related macular degeneration (nAMD) in 2020 and diabetic macular edema in 2022.[1–3] Brolucizumab is a humanized single-chain antibody fragment, which binds to all human isoforms of vascular endothelial growth factor A (VEGF-A).[4,5] Due to its small molecular size of 26 kDa, brolucizumab reaches 10–20 times higher molar concentration in comparison to aflibercept and ranibizumab, which may lead to a better penetration and therefore explain its greater reduction in the central retinal thickness and longer dosing intervals in the HAWK and HARRIER trials.[4–6] Albeit brolucizumab showed comparable outcome of visual acuity and superior results of central retinal thickness to other licensed anti-VEGF agents with an extended injection frequency, the safety concerns have been raised because of the increased number of retinal vasculitis (RV) and/or retinal vascular occlusion (RO) following intravitreal brolucizumab injection.[6–16] The incidence rate for intraocular inflammation (IOI) and RO with/without RV was reported between 2.0 and 2.4%, however, nearly a four times higher rate was observed in patients with RO

CONTACT B. Sobolewska bianka.sob@gmx.de Centre of Ophthalmology, Eberhard-Karls University, Elfriede-Aulhorn-Straße 7, 72076 Tuebingen, Germany
*Additional affiliation: Helmholtz Institute for Translational AngioCardioScience (HI-TAC), Mannheim, Germany.

compared to patients without a history of RO in the 12 months before brolucizumab treatment.[6,17,18] The Safety Review Committee (SRC) assessed an overall incidence rate of 3.3% for IOI and RV and 2.1% RO on the basis of investigator-reported adverse events in the phase 3 HAWK and HARRIER trials.[17] Such adverse events are associated with an increased risk of visual loss.[17] Therefore, fundamental research is mandatory to better understand the pathophysiological mechanisms. Until now, the potential role of anti-drug antibodies (ADAs) has been widely discussed, however, their presence does not seem to precondition brolucizumab-associated adverse events.[15,19] Lastly, Deissler et al. showed a destabilization of the barrier formed by immortalized bovine retinal endothelial cells (iBREC) at concentrations achievable by intravitreal injection of brolucizumab, which might be further potentiated by the surfactant polysorbate-80, leading to a higher systemic concentration of brolucizumab.[20] In this connection, endothelial dysfunction might activate platelets and results in retinal occlusion. Besides their essential role in homeostasis and vascular integrity, platelets are chief cells responding to vascular injury.[21] The endothelial disruption activates platelets leading to their adhesion, followed by the release of platelet granules such as VEGF or P-selectin, and activation of GPIIb/IIIa resulting in the formation of stable platelet aggregates.[22–24] After platelet stimulation, P-selectin is translocated from alfa-granules to the platelet external membrane leading to its increased surface expression. This crucial adhesion molecule binds to P-selectin glycoprotein ligand-1 (PSGL-1) on leukocytes providing an important link between the hemostatic and inflammatory responses.[25,26] The crosstalk between platelets and other cells is also associated with thrombotic dysregulation promoting venous and arterial thrombosis and prolonged inflammation in vasculitis.[27–29] Therefore, platelet activation and an increased expression of their surface activation markers might not only contribute to higher risk of vascular thrombosis such as myocardial infarction or stroke,[30,31] but could also play a crucial role in the pathophysiology of RV and/or RO following intravitreal brolucizumab injection. Whether brolucizumab may affect directly platelet activation or platelet reactivity to a platelet agonist remains unknown. Thus, the interactions of brolucizumab with platelets and an impact of brolucizumab on platelet activation profile were investigated in this study.

Materials and methods

Isolation of platelets

Platelets were obtained by centrifugating platelet-rich plasma (PRP) at 1000 rpm for 10 min at room temperature from venous blood, which was drawn into acid-citrate-dextrose (ACD) anticoagulant from healthy Caucasian volunteers, who did not have any ocular or systemic disease (n = 12; mean age: 35.67 (range: 26–55); eight women and four men). The volunteers had not taken any drugs during the last 10 days before the blood sampling. The number of platelets in the PRP was determined with a fully automated hematology analyzer and adjusted to 300 000 platelets/µL with platelet-poor plasma (PPP). This work adhered to the tenets of the Declaration of Helsinki and the Institutional Ethics Committee of the University of Tuebingen.

Brolucizumab and its solvent

Brolucizumab (Novartis Pharma GmbH, Nuremberg, Germany) was used in the following concentration range: 0.6 µg/mL, 3 µg/mL, 6 µg/mL, 300 µg/mL, and 3000 µg/mL. The chosen concentration range was decided on the basis of preclinical data on cynomolgus monkeys (6000 µg/eye with total of three intravitreal injections) and data of nAMD patients.[1] In cynomolgus monkeys, the mean serum cmax (maximal concentration) was approximately 0.3 µg/mL[5] and vitreous humor concentration was >1000-fold higher than those in the serum.[32] According to Novartis, the mean serum cmax was 50 ng/mL (range 8.97–548 ng/mL) in nAMD patients after intravitreal brolucizumab injection.[33]

Brolucizumab solvent was prepared according to the specifications of the manufacturer (10 mM sodium citrate, 0.02% polysorbate 80, 5.8% sucrose, pH 7.2). The prepared solvent did not contain any brolucizumab because it served as a control. The pH was set at 7.2 to be identical to brolucizumab solvent.

Antibodies

Activated glycoprotein (GP) IIb/IIIa (dilution: 1:20, PAC-1-FITC, BD Biosciences, San Jose, CA) and expression of P-selectin (dilution: 1:20, CD62P-FITC, Beckman Coulter, Marseille, France) are major surface activation markers and potential circulating platelet biomarkers indicating a high risk for future vascular thrombosis, therefore, they were chosen to investigate the impact of brolucizumab on platelet activation or reactivity as previously described.[34] The platelet population was identified by CD42b-PE (dilution: 1:20, Beckman Coulter, Marseille, France). FITC-labeled brolucizumab (dilution: 1:10, Brolucizumab; Novartis Pharma GmbH, Nuremberg, Germany) and FITC-labeled bevacizumab (dilution: 1:10, Avastin; Roche Pharma, Grenzach, Germany) were conducted according to the standard procedures provided by the manufacturer (Sigma Aldrich, St. Louis, MO). Antibodies against brolucizumab were used as primary antibody in a dilution of 1:10 000. Any change in the binding of antibodies was determined by flow cytometry.

Incubation of platelets with brolucizumab and activation of platelets

Platelets were exposed to brolucizumab or its solvent without brolucizumab, or phosphate-buffered saline (PBS) without brolucizumab. Both solvent without brolucizumab (because Beovu® contains brolucizumab including solvent) and PBS without brolucizumab served as two independent controls. The following concentrations of brolucizumab were used in platelets exposed to brolucizumab: 0.6 µg/mL, 3 µg/mL, 6 µg/mL, 300 µg/mL, and 3000 µg/mL. All samples with: (1) brolucizumab, and both controls: (2) solvent and (3) PBS were incubated for 30 min at room temperature. After incubation with brolucizumab or solvent or PBS,

thrombin receptor-activating peptide-6 (TRAP)-activated platelets (25 µM, Roche Diagnostics GmbH, Mannheim, Germany) was used to activate platelets. Thereafter, the following antibodies CD62P-FITC and PAC-1-FITC were added to the resting or activated platelets. As a control antibody, CD42b-PE was used to identify the platelet population in the whole blood. Subsequently, the samples were fixed with 0.05% paraformaldehyde in PBS as previously prescribed.[34]

Flow cytometry and multispectral imaging flow cytometry

Flow cytometry and Multispectral Imaging Flow Cytometry were performed on resting (non-activated) and activated platelets using the BD FACSCanto™ II Flow Cytometry Systems (BD Biosciences, Franklin Lakes, NJ) and ImageStream^{x} Mark II – multispectral imaging flow cytometer using the INSPIRE instrument controller software with 60× magnification and the 488 nm laser on 200mW (Cytek Biosciences/Amnis®, Seattle, WA), which is a combination of two methods: flow cytometry and fluorescence microscopy. The IDEAS® Image Analysis software was used for analysis of the ImageStream^{x} Mark II-data.

The specific monoclonal antibody binding was determined as percentage of gated platelets, and in drug-FITC as normalized MFI (mean fluorescent intensity; MFI^{FITC}+/$MFI^{total\ ratio}$).

Interaction between brolucizumab-FITC and platelets

A cross-pretreatment experiment was performed with brolucizumab and bevacizumab (bevacizumab Avastin; Roche, Grenzach, Germany). The concentrations of bevacizumab-FITC were chosen according to the FACS results on a dose-dependent platelet uptake of FITC-labeled bevacizumab by Verheul et al.[35] Platelets were incubated with PBS or brolucizumab (6 ng/mL, 600 ng/mL) or bevacizumab (2 µg/mL, 20 µg/mL) for 30 min followed by a cross-antibody staining (FITC-labeled brolucizumab or bevacizumab) and analyzed at the BD FACS Canto II. The optimal incubation time was determined by platelet analysis after single-agent pretreatment prior to the cross-treatment with brolucizumab for 15 min, 30 min, and 120 min followed by a brolucizumab-FITC staining. After incubation time for 30 min at room temperature, the antibodies were added and fixed with 0.05% paraformaldehyde in PBS.

Interaction between anti-brolucizumab antibodies generated by rabbit immunization and platelets

Brolucizumab was purified and analyzed by a high-pressure size exclusion chromatography with a FPLC Superdex S200 column 10/300GL in PBS pH 7.2 at a flow rate of 0.9 mL/min (MERCK, Darmstadt, Germany). Thereafter, the purified antigen was injected into a rabbit for custom generation of antibodies against brolucizumab by ProteoGenix (Schiltigheim, France). We followed the protocol according to Samara et al.[36] using three booster injections. After 80 days of immunization, a titer against brolucizumab was detectable with enzyme-linked immunosorbent assay up to 1:50 000. A working titer of 1:10 000 with a final concentration of 10 µg/mL was used. As a control, pre-immune rabbit serum was used. The platelets were pretreated for 30 min with 3 µg/mL, 300 µg/mL, and 3000 µg/mL brolucizumab or its solvent or PBS, followed by anti-brolucizumab antibody addition to resting and TRAP-activated platelets. The samples were fixed with 0.05% paraformaldehyde in PBS.

Statistical analysis

All data are presented as mean ± confidence interval (CI). To compare three or more groups, analysis of variance with Bonferroni's correction was performed. Each group was normally distributed and consisted of at least three experiments from different volunteers. A p value ≤.05 was considered statistically significant. GNU PSPP version 0.10.2-g654fff was used for statistical analyses.

Results

Platelet activation profile in non-activated (resting) platelets

The expression of both surface activation markers CDP2P and GPIIb/IIIa did not significantly differ between brolucizumab 0.6 µg/mL, 3 µg/mL, 6 µg/mL, 300 µg/mL, and 3000 µg/mL and its solvent or PBS (CD62P: P between all groups = 0.530, GPIIb/IIIa: P between all groups = 0.052) (Figures 1(A,B), 2(A,C), and 3(A–D)). The data are provided in Table 1.

Platelet activation profile in activated platelets

The expression of TRAP-activated CD62P after preincubation with brolucizumab 0.6 µg/mL, 3 µg/mL, 6 µg/mL, 300 µg/mL, and 3000 µg/mL compared to solvent or PBS (P between all groups = 0.767) (Figures 1(C), 2(B), and 3(A′,B′)). The data are shown in Table 1.

No significant upregulation of TRAP-activated GPIIb/IIIa was found after following brolucizumab concentrations 0.6 µg/mL, 3 µg/mL, 6 µg/mL, 300 µg/mL, and 3000 µg/mL compared to solvent or PBS (P between all groups = 0.727) (Figures 1(D), 2(D), and 3(C′,D′)). The data are provided in Table 1.

Interaction between brolucizumab-FITC and platelets

Brolucizumab-FITC (with PBS) was significantly downregulated in comparison to bevacizumab-FITC (normalized MFI = 3.32, CI: 3.16–3.48 vs. normalized MFI = 7.19, CI: 7.04–7.35; p < .001) (Figure 4). A significant difference was observed between brolucizumab-FITC without (with PBS) and with pretreatment with brolucizumab or bevacizumab (p < .001) (Table 2). Bevacizumab-FITC was downregulated after pretreatment with brolucizumab or bevacizumab compared to bevacizumab-FITC without pretreatment (with PBS) (p < .001) (Table 2). In the post hoc analysis, there was no significant difference regarding the pretreatment with brolucizumab (6 ng/mL, 600 ng/mL) or bevacizumab (2 µg/

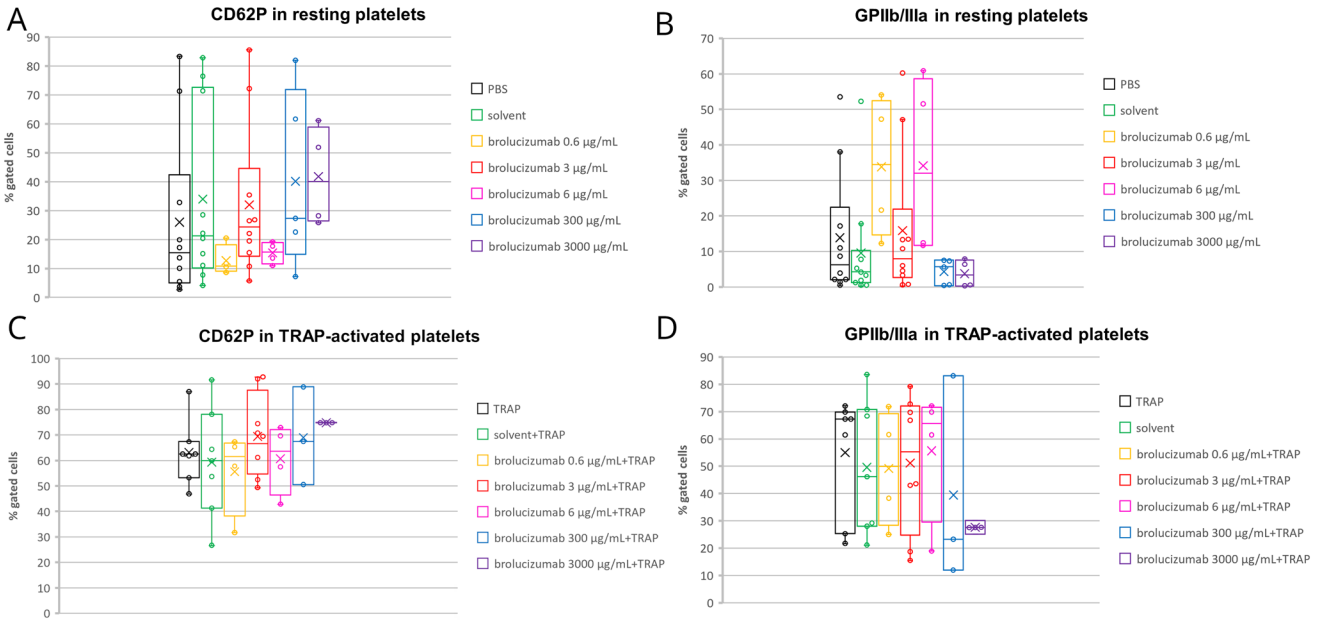

Figure 1. CD62P and GPIIb/IIIa in resting and TRAP-activated platelets after exposure to brolucizumab. Box plot showing the percentage of gated platelets. The expression of CD62P (A, C) and GPIIb/IIIa (B, D) after exposure to brolucizumab 0.6 µg/mL, 3 µg/mL, and 6 µg/mL, 300 µg/mL, and 3000 µg/mL its solvent, and PBS in resting and activated platelets. No significant upregulation of CD62P or GPIIb/IIIa between all brolucizumab concentrations and its solvent or PBS (phosphate-buffered saline).

Table 1. The percentage of gated platelets with surface expression of CD62P and GPIIb/IIIa in resting and TRAP-activated platelets (CI: confidence interval).

Platelet surface activation marker	Mean (CI) in resting platelets	Mean (CI) in TRAP-activated platelets
CD62P		
PBS	26.01 (5.53–46.50)	4.60 (3.18–6.01)
Solvent	33.99 (12.12–55.87)	4.60 (3.22–6.04)
Brolucizumab 0.6 µg/mL	12.71 (4.25–21.18)	4.48 (3.22–55.74)
Brolucizumab 3 µg/mL	31.99 (13.17–50.81)	5.67 (3.29–8.05)
Brolucizumab 6 µg/mL	15.40 (9.27–21.53)	4.32 (3.46–5.18)
Brolucizumab 300 µg/mL	40.16 (2.02–78.30)	4.66 (−2.52 to 11.83)
Brolucizumab 3000 µg/mL	41.77 (13.92–69.63)	3.62 (2.04–5.19)
GPIIb/IIIa		
PBS	13.85 (0.99–26.72)	55.02 (34.82–75.21)
Solvent	9.57 (−1.79 to 20.92)	49.57 (26.66–72.47)
Brolucizumab 0.6 µg/mL	33.84 (1.94–65.74)	49.20 (15.20–83.20)
Brolucizumab 3 µg/mL	15.89 (1.08–30.70)	51.18 (30.44–71.92)
Brolucizumab 6 µg/mL	34.13 (−7.05 to 75.32)	55.63 (15.98–95.28)
Brolucizumab 300 µg/mL	4.31 (−0.28 to 8.89)	39.47 (−55.45 to 134.38)
Brolucizumab 3000 µg/mL	3.73 (−2.72 to 10.18)	27.60 (21.39–33.81)

mL, 20 µg/mL) both within and between brolucizumab-FITC and bevacizumab-FITC (Figure 4).

Interaction between antibodies against brolucizumab and platelets

The percentage of gated platelets with antibodies against brolucizumab did not show any statistical difference between different brolucizumab concentrations (3 µg/mL, 300 µg/mL, and 3000 µg/mL) and its solvent in resting and TRAP-activated platelets (p = .602 and p = .824, respectively) (Table 3, Figures 2(E,F) and 5).

Discussion

The reported IOIs with vascular involvement had not been described in this frequency and severity for other intravitreally administered VEGF inhibitors. Since patients with brolucizumab-associated RAO within the HARRIER and HAWK trials had cardiovascular comorbidities such as hypertension and cardial arrhythmias[37] and platelets are affected by the presence of cardiovascular risk factors or established cardiovascular diseases or aging-related diseases,[38–40] risk factors for RO have to be considered. P-selectin or the platelet glycoprotein IIb/IIIa (GPIIb/IIIa) receptor play a key role in the pathway of thrombus formation.[26,41,42] Cardiovascular risk factors can trigger and alter platelet function by circulating oxLDL,[39] cytokines,[43] abnormal blood flow, vascular wall health status such as endothelial dysfunction or damage or hematological factors.[44] Brolucizumab was investigated in both non-activated and activated platelets using conventional flow cytometry and multispectral imaging flow cytometry, combining the phenotyping abilities of flow cytometry with a high-resolution fluorescence microscopy. In this study, no effect of brolucizumab on resting and activated platelets was found. TRAP-induced activation of the fibrinogen receptor GPIIb/IIIa and P-selectin pretreated with brolucizumab did not show any significant upregulation in comparison to brolucizumab solvent or untreated platelets. With increasing brolucizumab concentrations (0.6 µg/mL, 3 µg/mL, 6 µg/mL, 300 µg/mL, and 3000 µg/mL), no impact on the platelet activation profile was observed. To further clarify the interaction between brolucizumab and platelets, a cross-pretreatment experiment with brolucizumab and bevacizumab was performed to exclude a concentration-dependent interaction of brolucizumab with platelets.[35,45,46] Here, a significantly higher binding capacity of bevacizumab-FITC was shown compared to brolucizumab-FITC, which can be explained due to their different molecular structures. Given that bevacizumab contains a Fc region and colocalized with clathrin-coated pits in

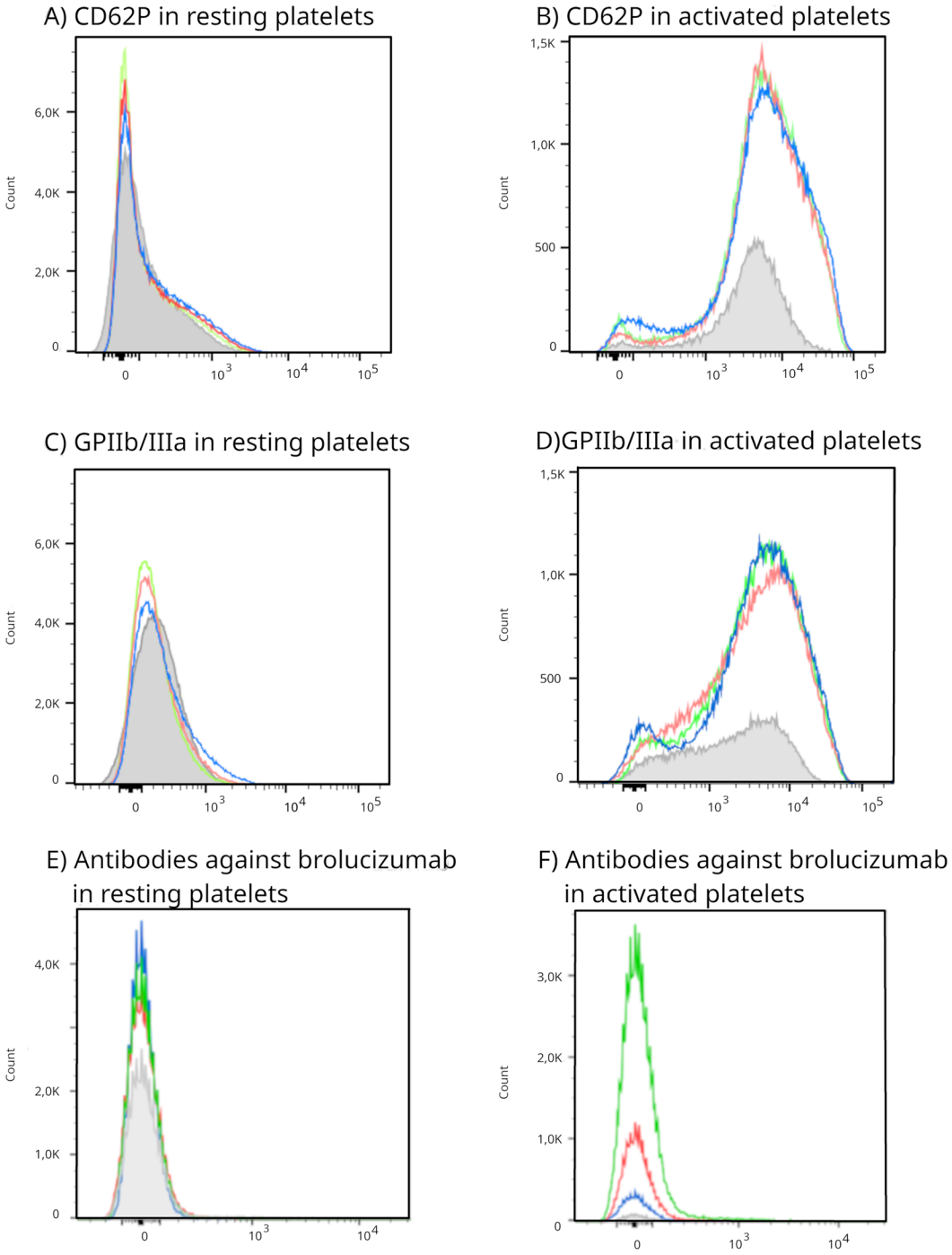

Figure 2. Overlay histogram of CD62P (A, B), GPIIb/IIIa (C, D), and antibodies against brolucizumab (E, F) in resting and TRAP-activated platelets after exposure to PBS (grey), solvent (green), 3 µg/mL brolucizumab (red), and 300 µg/mL brolucizumab (blue).

platelets, Fc-receptor-dependent endocytosis was suggested.[46] Moreover, pretreatment with bevacizumab showed a significant downregulation of bevacizumab-FITC compared to bevacizumab-FITC with PBS, which may indicate occupation of Fc receptors on platelets by pretreated bevacizumab. These findings are in accordance with other studies on binding mechanisms of bevacizumab.[46–48] In addition, the cross-pretreatment experiment revealed a significantly weaker brolucizumab-FITC signal after exposure to both brolucizumab and bevacizumab. For further explanation of brolucizumab binding on platelets brolucizumab-associated AEs, antibodies against brolucizumab from immunized rabbits were prepared. However, no interaction was found after pretreatment with different brolucizumab-induced autoantibody concentrations compared to solvent in resting or activated platelets. Since we did not provide evidence for the binding capacity of brolucizumab, the significant downregulation of FITC-brolucizumab after exposure to brolucizumab or bevacizumab may imply that brolucizumab is taken-up in platelets, and therefore, eliminated or released in higher concentrations at the inflammatory sites leading to thromboinflammation in patients at risk of brolucizumab-associated

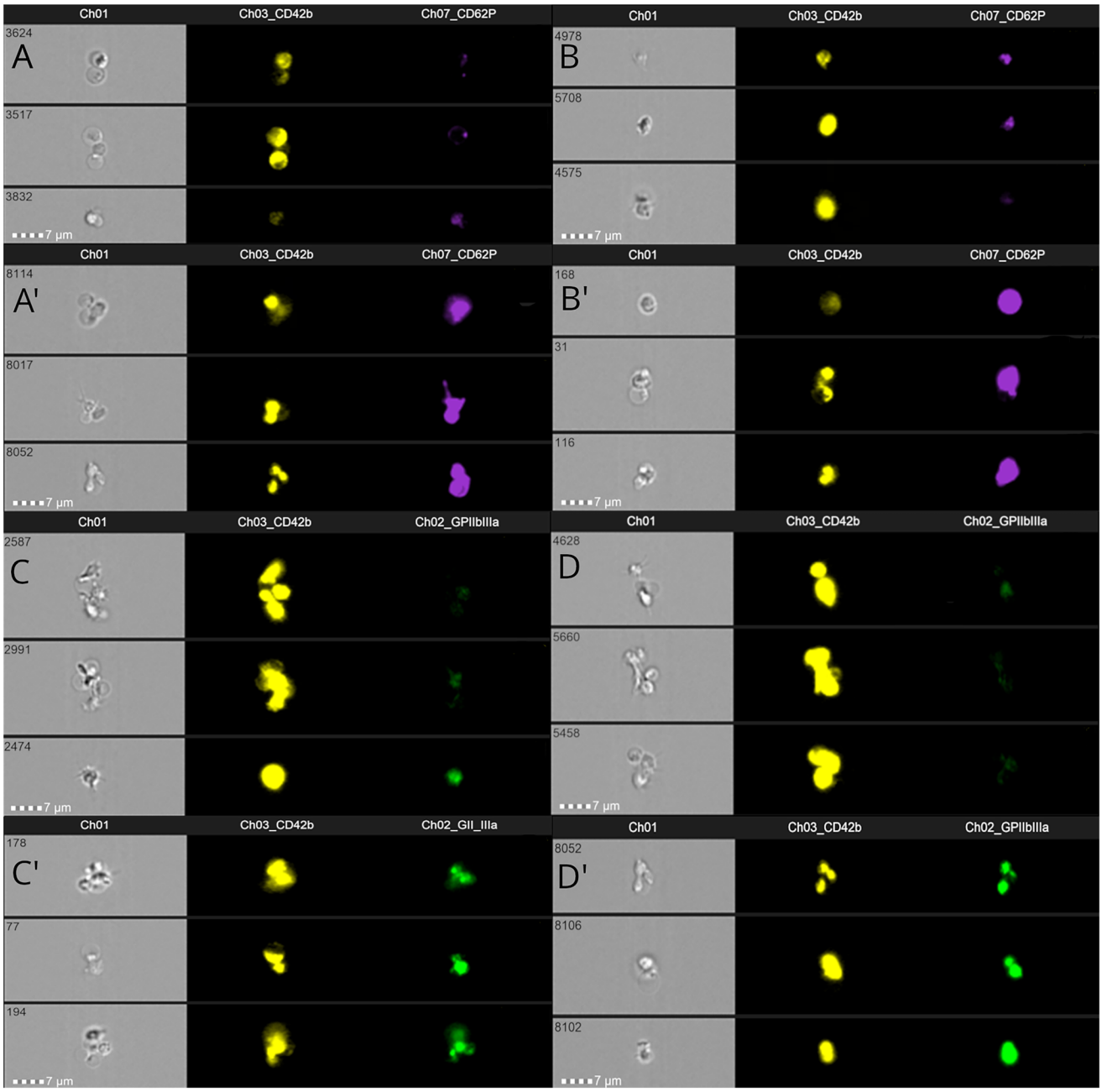

Figure 3. Representative cell images in resting and activated platelets after exposure to brolucizumab 300 µg/mL and its solvent. CD62P expression after pretreatment with brolucizumab 300 µg/mL in resting (A) and activated platelets (A′), solvent in resting (B) and activated platelets (B′). GPIIb/IIIa expression after pretreatment with brolucizumab 300 µg/mL in resting (C) and activated platelets (C′), solvent in resting (D) and activated platelets (D′).

adverse events, for example: female patients, diabetic patients, patients with cardiovascular diseases.[15,16,19,23,37] Several platelet transporters as carriers for a wide spectrum of substrates, including inorganic ions, amino acids, nucleotides, and drugs were identified. Although it is well known that platelets are so called "long-haul truckers" and play an important role in pharmacotherapy such as drug side effects due to storage and elimination of drugs, their function is not fully characterized.[49]

At first brolucizumab-related AEs were linked with ADAs to brolucizumab, which as immune complexes deposited on retinal walls leading to RV with or without RO.[8,15,19,50] Beside the type III hypersensitivity reaction (HSR),[8,15,16] delayed type IV (T-cell mediated) HSR might contribute to development of brolucizumab-related AEs,[15,16] since it is known that other therapeutics, for example, monoclonal antibodies or antibiotics, may provoke diverse adverse events including all types of hypersensitivities.[51,52] Moreover, the post hoc analysis of the HAWK and HARRIER studies revealed that approximately half of cases of brolucizumab-AE occurred 3 months after the first injection, with a considerable higher number of cases after multiple injections.[17] Such delayed immunological responses could be triggered by the degradation products of brolucizumab or the VEGF–brolucizumab complex.

Currently, other mechanisms of pathogenesis than adaptive immune responses are suggested[15] since the presence of ADAs

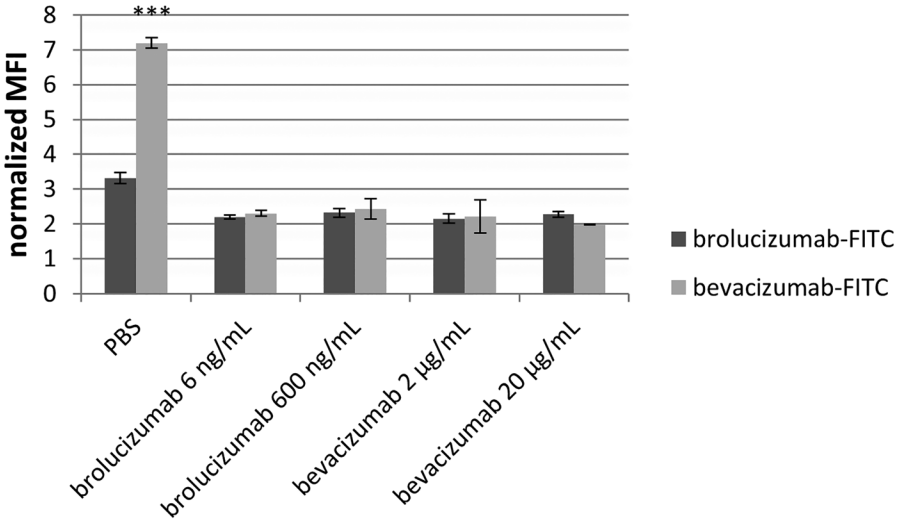

Figure 4. Brolucizumab-FITC and bevacizumab-FITC as IgG control. Less binding of brolucizumab-FITC in comparison to bevacizumab-FITC was observed in untreated platelets (***$p < .001$) without significant difference regarding the pretreatment with brolucizumab or bevacizumab both within and between brolucizumab-FITC and bevacizumab-FITC; normalized MFI – mean fluorescent intensity.

Table 2. Normalized MFI (mean fluorescent intensity) of brolucizumab-FITC and bevacizumab-FITC after pretreatment with PBS, brolucizumab 6 ng/mL, brolucizumab 600 ng/mL, bevacizumab 2 µg/mL, and bevacizumab 20 µg/mL (CI: confidence interval).

FITC labeled drug	Mean (CI)	*p* Value
Brolucizumab-FITC		
PBS	3.22 (2.12–4.33)	$p < .001$
Brolucizumab 6 ng/mL	2.19 (2.14–2.25)	
Brolucizumab 600 ng/mL	2.32 (2.19–2.44)	
Bevacizumab 2 µg/mL	2.15 (2.02–2.29)	
Bevacizumab 20 µg/mL	2.27 (2.18–2.35)	
Bevacizumab-FITC		
PBS	7.19 (7.04–7.35)	$p < .001$
Brolucizumab 6 ng/mL	2.30 (2.22–2.39)	
Brolucizumab 600 ng/mL	2.43 (2.14–2.72)	
Bevacizumab 2 µg/mL	2.21 (1.74–2.68)	
Bevacizumab 20 µg/mL	1.98 (1.96–1.99)	

Table 3. The percentage of gated platelets with antibodies against brolucizumab in resting and TRAP-activated platelets (CI: confidence interval).

Antibodies against brolucizumab	Mean (CI) in resting platelets	Mean (CI) in TRAP-activated platelets
PBS	1.44 (−0.57 to 3.45)	0.53 (0.21–0.85)
Solvent	1.01 (0.33–1.69)	1.11 (−2.45 to 4.66)
Brolucizumab 3 µg/mL	0.97 (0.17–1.78)	1.10 (−1.86 to 4.07)
Brolucizumab 300 µg/mL	0.81 (0.38–1.25)	0.63 (−0.06 to 1.32)
Brolucizumab 3000 µg/mL	0,51 (0.14–0.89)	0.54 (0.29–0.79)

does not precondition brolucizumab-associated AEs.[19,53] The first *in vitro* proof for an alternative pathophysiology of brolucizumab-related AEs was provided by Deissler et al.[20] Strictly speaking, they showed lower levels of claudin-1, which is an essential part of the iBREC barrier.[20] These findings support the hypothesis of breakdown of the inner-blood–retinal barrier, which was postulated by Haug et al.[13] They hypothesized that intravitreal high concentration of brolucizumab in diabetic patients or any prior brolucizumab-related AE may breakdown the inner-blood–retinal barrier and disrupt Anterior Chamber-Associated Immune Deviation (ACAID) resulting in exaggerated inflammatory responses.[13,54] For example, tumor necrosis factor-alpha (TNF-α) or IL-17A contributes to platelet activation followed by P-selectin external membrane translocation facilitating rolling of platelets on activated endothelial cells.[55] P-selectin also binds to glycoprotein ligand 1 (PSGL1) of circulating monocytes forming monocyte–platelet aggregates (MPAs).[38] Both platelet-bound and soluble P-selectin increased TF (tissue factor) expression on monocytes resulting in fibrin generation and clot formation by bridging GPIIb/IIIa between platelets.[56–59] Further, activated platelets contribute to inflammatory processes by release of alpha and dense granules, shedding microvesicles, and mediating adhesion to monocytes via P-selectin glycoprotein 1 (PSGL-1) axis enhancing secretion of inflammatory cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, IL-8, IL-12, and macrophage inflammatory protein-1β.[43,56,60,61] Although this study did not show any significant upregulation of platelet activation markers after exposure to increasing brolucizumab concentrations, the platelet activation profile of gated platelets differed between subjects showing greater ranges, especially in higher brolucizumab concentrations (300 µg/mL and 3000 µg/mL). Here, a higher P-selectin and a lower GPIIb/IIIA surface expressions were observed, however, without statistical significance. In addition to the P-selectin's role in platelet aggregation facilitating the formation of large stable platelet aggregates,[26] it mediates platelet–leucocyte interactions in thrombosis and inflammation.[29] Therefore, its higher expression might favor priming of platelets, but not necessarily lead to their aggregation, and reinforce vascular occlusion. Nevertheless, a connection would be in line with an increased risk in female patients for brolucizumab-associated events[15,16,62] in accordance to sex differences in platelet reactivity.[63] Their increased responsiveness is explained by a higher tendency to activate GPIIb/IIIa and/or P-selectin leading to enhanced prothrombotic and proinflammatory function of platelets.[63–68] Lastly, it has to be pointed out that brolucizumab-associated AEs also vary in manifestation and extent between patients, from possibly overlooked to severe AEs with deterioration of visual acuity.[15,17]

Since brolucizumab-exposed iBREC did not secrete any inflammatory cytokines,[20] platelets might be cellular mediators

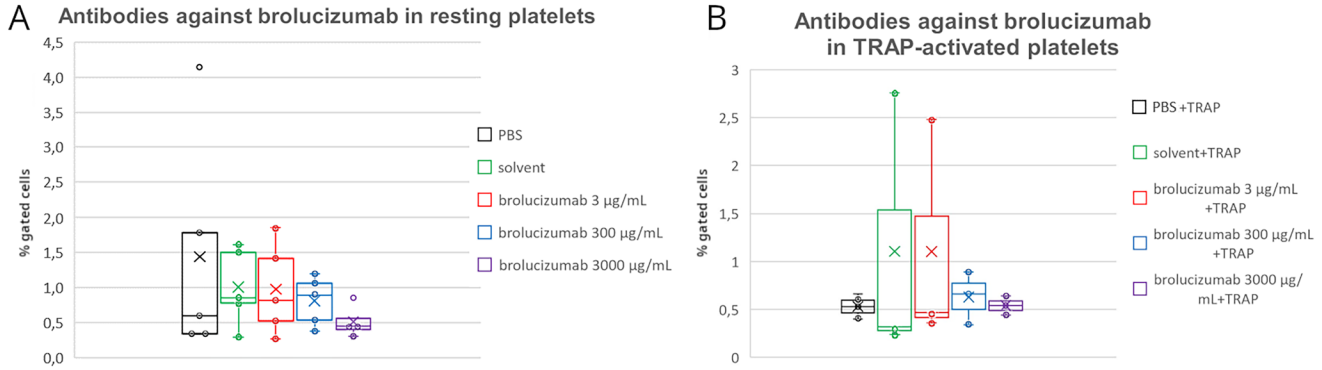

Figure 5. Antibodies against brolucizumab in resting and TRAP-activated platelets. Box plot showing the percentage of gated platelets. No significant upregulation of antibodies against brolucizumab in resting (A) and TRAP-activated platelets (B).

of thrombosis and inflammation. We therefore hypothesize that destabilization of the inner-blood-retinal barrier due to brolucizumab might be potentiated due to disruption of ACAID leading to activation of inflammation and/or platelets, which can manifest as IOI and/or RV and/or RO.[13,20,52,55–59] Important limitations must be considered: our experiments were limited to: (1) *in vitro* experiments, (2) platelets without analysis of innate and cellular immune responses, (3) a certain sample size, and (4) and higher female:male ratio. However, female patients may be more prone to brolucizumab-related adverse events.[15,16,62] Platelets from healthy donors were used and not cells from elderly patients with retinal disease. The absence of corresponding activation in the Petri dish does not rule out the possibility that the drug molecules could trigger other reactions in the local tissue environment with drug-related antibodies and high VEGF levels.

In conclusion, our findings did not show any significantly increased expression of GPIIb/IIIa and P-selectin (CD62P) after exposure to increasing concentrations of brolucizumab in resting and activated platelets. No platelet interactions were observed with anti-brolucizumab antibodies. Given that brolucizumab-FITC was downregulated after pretreatment with brolucizumab, an uptake of brolucizumab in platelets is likely. Thus, future research on underlying mechanisms of brolucizumab-associated adverse events is required.

Author contributions

All authors contributed to all of the following: (1) conception and design of the work, acquisition of data, or analysis and interpretation of data, (2) drafting the article or revising it critically for important intellectual content, (3) final approval of the version to be published, and (4) agreement to be accountable for all aspects of the work.

Disclosure statement

B. Sobolewska has received one travel grant from Galderma, Novartis, Santen Pharmaceutical Co., and Bausch & Lomb.
F. Ziemssen has received consulting fees from Alimera, Allergan, Bayer HealthCare, and Novartis, and speaker fees from Alcon, Alimera, Allergan, Bayer HealthCare, and Novartis.
Konstantinos Stellos is supported by grants from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (MODVASC, Grant Agreement No. 759248), the German Research Foundation DFG (CRC1366 C07, Project Number 394046768), the Health + Life Science Alliance Heidelberg Mannheim GmbH, and the Biotechnology and Biological Sciences Research Council (BBSRC) of the UK Research and Innovation (UKRI).

ORCID

F. Ziemssen http://orcid.org/0000-0002-3873-0581

Data availability statement

The authors confirm that the data supporting the findings of this study are available within the article [and/or] its supplementary materials.

References

1. Dugel PU, Koh A, Ogura Y, Jaffe GJ, Schmidt-Erfurth U, Brown DM, Gomes AV, Warburton J, Weichselberger A, Holz FG, et al. HAWK and HARRIER: phase 3, multicenter, randomized, double-masked trials of brolucizumab for neovascular age-related macular degeneration. Ophthalmology. 2020;127(1):72–84. doi: 10.1016/j.ophtha.2019.04.017.
2. Brown DM, Emanuelli A, Bandello F, Barranco JJE, Figueira J, Souied E, Wolf S, Gupta V, Ngah NF, Liew G, et al. KESTREL and KITE: 52-week results from two phase III pivotal trials of brolucizumab for diabetic macular edema. Am J Ophthalmol. 2022;238:157–172. doi: 10.1016/j.ajo.2022.01.004.
3. Holz FG, Dugel PU, Weissgerber G, Hamilton R, Silva R, Bandello F, Larsen M, Weichselberger A, Wenzel A, Schmidt A, et al. Single-chain antibody fragment VEGF inhibitor RTH258 for neovascular age-related macular degeneration: a randomized controlled study. Ophthalmology. 2016;123(5):1080–1089. doi: 10.1016/j.ophtha.2015.12.030.
4. Tietz J, Schmid G, Konrad J, Jampen S, Maurer P, Schmidt A, Escher D. Affinity and potency of RTH258 (ESBA1008), a novel inhibitor of vascular endothelial growth factor A for the treatment of retinal disorders. Invest Ophthalmol Vis Sci. 2015;56:1501.
5. Gaudreault J, Gunde T, Floyd HS, Ellis J, Tietz J, Binggeli D, Keller B, Schmidt A, Escher D. Preclinical pharmacology and safety of ESBA1008, a single-chain antibody fragment, investigated as potential treatment for age related macular degeneration. Poster presented at: the Annual Meeting of the Association for Research in Vision and Ophthalmology (ARVO); 2012 May 6–10; Fort Lauderdale, Florida.
6. Nguyen QD, Das A, Do DV, Dugel PU, Gomes A, Holz FG, Koh A, Pan CK, Sepah YJ, Patel N, et al. Brolucizumab: evolution through preclinical and clinical studies and the implications for the management of neovascular age-related macular degeneration. Ophthalmology. 2020;127(7):963–976. doi: 10.1016/j.ophtha.2019.12.031.

7. Finger RP, Dennis N, Freitas R, Quenéchdu A, Clemens A, Karcher H, Souied EH. Comparative efficacy of brolucizumab in the treatment of neovascular age-related macular degeneration: a systematic literature review and network meta-analysis. Adv Ther. 2022;39(8):3425–3448. doi: 10.1007/s12325-022-02193-3.
8. Sharma A, Kumar N, Parachuri N, Sharma R, Bandello F, Kuppermann BD, Loewenstein A. Brolucizumab and immunogenicity. Eye. 2020;34(10):1726–1728. doi: 10.1038/s41433-020-0853-9.
9. Baumal CR, Bodaghi B, Singer M, Tanzer DJ, Seres A, Joshi MR, Feltgen N, Gale R. Expert opinion on management of intraocular inflammation, retinal vasculitis, and vascular occlusion after brolucizumab treatment. Ophthalmol Retina. 2021;5(6):519–527. doi: 10.1016/j.oret.2020.09.020.
10. Khanani AM, Zarbin MA, Barakat MR, Albini TA, Kaiser PK, Guruprasad B, Agashivala N, Yu JS, Wykoff CC, MacCumber MW. Safety outcomes of brolucizumab in neovascular age-related macular degeneration: results from the IRIS Registry and Komodo Healthcare Map. JAMA Ophthalmol. 2022;140(1):20–28. doi: 10.1001/jamaophthalmol.2021.4585.
11. Maruko I, Okada AA, Iida T, Hasegawa T, Izumi T, Kawai M, Maruko R, Nakayama M, Yamamoto A, Koizumi H, et al. Brolucizumab-related intraocular inflammation in Japanese patients with age-related macular degeneration: a short-term multicenter study. Graefes Arch Clin Exp Ophthalmol. 2021;259(9):2857–2859. doi: 10.1007/s00417-021-05136-w.
12. Iyer PG, Albini TA. Drug-related adverse effects of antivascular endothelial growth factor agents. Curr Opin Ophthalmol. 2021;32(3):191–197. doi: 10.1097/ICU.0000000000000757.
13. Haug SJ, Hien DL, Uludag G, Ngoc TTT, Lajevardi S, Halim MS, Sepah YJ, Do DV, Khanani AM. Retinal arterial occlusive vasculitis following intravitreal brolucizumab administration. Am J Ophthalmol Case Rep. 2020;18:100680. doi: 10.1016/j.ajoc.2020.100680.
14. Jain A, Chea S, Matsumiya W, Halim MS, Yaşar Ç, Kuang G, Sepah YJ, Khanani AM, Do DV, Nguyen QD. Severe vision loss secondary to retinal arteriolar occlusions after multiple intravitreal brolucizumab administrations. Am J Ophthalmol Case Rep. 2020;18:100687. doi: 10.1016/j.ajoc.2020.100687.
15. Witkin AJ, Hahn P, Murray TG, Arevalo JF, Blinder KJ, Choudhry N, Emerson GG, Goldberg RA, Kim SJ, Pearlman J, et al. Occlusive retinal vasculitis following intravitreal brolucizumab. J Vitreoretin Dis. 2020;4(4):269–279. doi: 10.1177/2474126420930863.
16. Baumal CR, Spaide RF, Vajzovic L, Freund KB, Walter SD, John V, Rich R, Chaudhry N, Lakhanpal RR, Oellers PR, et al. Retinal vasculitis and intraocular inflammation after intravitreal injection of brolucizumab. Ophthalmology. 2020;127(10):1345–1359. doi: 10.1016/j.ophtha.2020.04.017.
17. Monés J, Srivastava SK, Jaffe GJ, Tadayoni R, Albini TA, Kaiser PK, Holz FG, Korobelnik JF, Kim IK, Pruente C, et al. Risk of inflammation, retinal vasculitis, and retinal occlusion-related events with brolucizumab: post hoc review of HAWK and HARRIER. Ophthalmology. 2021;128(7):1050–1059. doi: 10.1016/j.ophtha.2020.11.011.
18. Khanani AM, Brown DM, Jaffe GJ, Wykoff CC, Adiguzel E, Wong R, Meng X, Heier JS, MERLIN Investigators. MERLIN: phase 3a, multicenter, randomized, double-masked trial of brolucizumab in participants with neovascular age-related macular degeneration and persistent retinal fluid. Ophthalmology. 2022;129(9):974–985. doi: 10.1016/j.ophtha.2022.04.028.
19. Busch M, Pfeil JM, Dähmcke M, Brauckmann T, Großjohann R, Chisci V, Hunfeld E, Eilts S, Omran W, Morawiec-Kisiel E, et al. Anti-drug antibodies to brolucizumab and ranibizumab in serum and vitreous of patients with ocular disease. Acta Ophthalmol. 2022;100(8):903–910. doi: 10.1111/aos.15124.
20. Deissler HL, Busch C, Wolf A, Brauckmann T, Großjohann R, Chisci V, Hunfeld E, Eilts S, Omran W, Morawiec-Kisiel E, et al. Beovu, but not Lucentis impairs the function of the barrier formed by retinal endothelial cells in vitro. Sci Rep. 2022;12(1):12493. doi: 10.1038/s41598-022-16770-7.
21. Rondina MT, Weyrich AS, Zimmerman GA. Platelets as cellular effectors of inflammation in vascular diseases. Circ Res. 2013;112(11):1506–1519. doi: 10.1161/CIRCRESAHA.113.300512.
22. Huang J, Li X, Shi X, Zhu M, Wang J, Huang S, Huang X, Wang H, Li L, Deng H, et al. Platelet integrin αIIbβ3: signal transduction, regulation, and its therapeutic targeting. J Hematol Oncol. 2019;12(1):26. doi: 10.1186/s13045-019-0709-6.
23. Qiao J, Wu X, Luo Q, Wei G, Xu M, Wu Y, Liu Y, Li X, Zi J, Ju W, et al. NLRP3 regulates platelet integrin αIIbβ3 outside-in signaling, hemostasis and arterial thrombosis. Haematologica. 2018;103(9):1568–1576. doi: 10.3324/haematol.2018.191700.
24. Huilcaman R, Venturini W, Fuenzalida L, Cayo A, Segovia R, Valenzuela C, Brown N, Moore-Carrasco R. Platelets, a key cell in inflammation and atherosclerosis progression. Cells. 2022;11(6):1014. doi: 10.3390/cells11061014.
25. Perkins LA, Anderson CJ, Novelli EM. Targeting P-selectin adhesion molecule in molecular imaging: p-selectin expression as a valuable imaging biomarker of inflammation in cardiovascular disease. J Nucl Med. 2019;60(12):1691–1697. doi: 10.2967/jnumed.118.225169.
26. Merten M, Thiagarajan P. P-selectin expression on platelets determines size and stability of platelet aggregates. Circulation. 2000;102(16):1931–1936. doi: 10.1161/01.cir.102.16.1931.
27. Kolarova H, Klinke A, Kremserova S, Adam M, Pekarova M, Baldus S, Eiserich JP, Kubala L. Myeloperoxidase induces the priming of platelets. Free Radic Biol Med. 2013;61:357–369. doi: 10.1016/j.freeradbiomed.2013.04.014.
28. Miao D, Li DY, Chen M, Zhao MH. Platelets are activated in ANCA-associated vasculitis via thrombin-PARs pathway and can activate the alternative complement pathway. Arthritis Res Ther. 2017;19(1):252. doi: 10.1186/s13075-017-1458-y.
29. Wienkamp AK, Erpenbeck L, Rossaint J. Platelets in the NETworks interweaving inflammation and thrombosis. Front Immunol. 2022;13:953129. doi: 10.3389/fimmu.2022.953129.
30. Hally KE, Parker OM, Brunton-O'Sullivan MM, Harding SA, Larsen PD. Linking neutrophil extracellular traps and platelet activation: a composite biomarker score for predicting outcomes after acute myocardial infarction. Thromb Haemost. 2021;121(12):1637–1649. doi: 10.1055/s-0041-1728763.
31. Yilmaz G, Granger DN. Cell adhesion molecules and ischemic stroke. Neurol Res. 2008;30(8):783–793. doi: 10.1179/174313208X341085.
32. Tadayoni R, Sararols L, Weissgerber G, Verma R, Clemens A, Holz FG. Brolucizumab: a newly developed anti-VEGF molecule for the treatment of neovascular age-related macular degeneration. Ophthalmologica. 2021;244(2):93–101. doi: 10.1159/000513048.
33. Beovu. European Medicines Agency; 2024. europa.eu.
34. Sobolewska B, Golenko J, Poeschel S, Grimmel C, Gatsiou A, Sopova K, Biedermann T, Schenke-Layland K, Stellos K, Ziemssen F. Influence of aflibercept on platelet activation profile. Exp Eye Res. 2018;175:166–172. doi: 10.1016/j.exer.2018.06.009.
35. Verheul HM, Lolkema MP, Qian DZ, Hilkes YH, Liapi E, Akkerman JW, Pili R, Voest EE. Platelets take up the monoclonal antibody bevacizumab. Clin Cancer Res. 2007;13(18 Pt 1):5341–5347. doi: 10.1158/1078-0432.CCR-07-0847.
36. Samara P, Kalbacher H, Ioannou K, Radu DL, Livaniou E, Promponas VJ, Voelter W, Tsitsilonis O. Development of an ELISA for the quantification of the C-terminal decapeptide prothymosin α(100–109) in sera of mice infected with bacteria. J Immunol Methods. 2013;395(1–2):54–62. doi: 10.1016/j.jim.2013.06.011.
37. Dugel PU, Singh RP, Koh A, Ogura Y, Weissgerber G, Gedif K, Jaffe GJ, Tadayoni R, Schmidt-Erfurth U, Holz FG. HAWK and HARRIER: ninety-six-week outcomes from the phase 3 trials of brolucizumab for neovascular age-related macular degeneration. Ophthalmology. 2021;128(1):89–99. doi: 10.1016/j.ophtha.2020.06.028.
38. Htun P, Fateh-Moghadam S, Tomandl B, Handschu R, Klinger K, Stellos K, Garlichs C, Daniel W, Gawaz M. Course of platelet activation and platelet–leukocyte interaction in cerebrovascular ischemia. Stroke. 2006;37(9):2283–2287. doi: 10.1161/01.STR.0000236638.75591.61.
39. Stellos K, Sauter R, Fahrleitner M, Grimm J, Stakos D, Emschermann F, Panagiota V, Gnerlich S, Perk A, Schönberger T, et al. Binding of oxidized low-density lipoprotein on circulating platelets is increased in patients with acute coronary syndromes and induces platelet adhesion to vascular wall in vivo – brief report. Arterioscler Thromb Vasc Biol. 2012;32(8):2017–2020. doi: 10.1161/ATVBAHA.111.244707.

40. Mukai R, Matsumoto H, Akiyama H. Risk factors for emerging intraocular inflammation after intravitreal brolucizumab injection for age-related macular degeneration. PLOS One. 2021;16(12):e0259879. doi: 10.1371/journal.pone.0259879.
41. Lordan R, Tsoupras A, Zabetakis I. Platelet activation and prothrombotic mediators at the nexus of inflammation and atherosclerosis: potential role of antiplatelet agents. Blood Rev. 2021;45:100694. doi: 10.1016/j.blre.2020.100694.
42. Stellos K, Panagiota V, Kögel A, Leyhe T, Gawaz M, Laske C. Predictive value of platelet activation for the rate of cognitive decline in Alzheimer's disease patients. J Cereb Blood Flow Metab. 2010;30(11):1817–1820. doi: 10.1038/jcbfm.2010.140.
43. Gatsiou A, Sopova K, Tselepis A, Stellos K. Interleukin-17A triggers the release of platelet-derived factors driving vascular endothelial cells toward a pro-angiogenic state. Cells. 2021;10(8):1855. doi: 10.3390/cells10081855.
44. Shin ES, Sorenson CM, Sheibani N. Diabetes and retinal vascular dysfunction. J Ophthalmic Vis Res. 2014;9(3):362–373. doi: 10.4103/2008-322X.143378.
45. Sobolewska B, Grimmel C, Gatsiou A, Sopova K, Klein J, Biedermann T, Stellos K, Ziemssen F. Different effects of ranibizumab and bevacizumab on platelet activation profile. Ophthalmologica. 2015;234(4):195–210. doi: 10.1159/000437057.
46. Sobolewska B, Fehrenbacher B, Münzer P, Kalbacher H, Geue S, Stellos K, Schaller M, Ziemssen F. Human platelets take up anti-VEGF agents. J Ophthalmol. 2021;2021:8811672. doi: 10.1155/2021/8811672.
47. Meyer T, Robles-Carrillo L, Robson T, Langer F, Desai H, Davila M, Amaya M, Francis JL, Amirkhosravi A. Bevacizumab immune complexes activate platelets and induce thrombosis in FCGR2A transgenic mice. J Thromb Haemost. 2009;7(1):171–181. doi: 10.1111/j.1538-7836.2008.03212.x.
48. Nomura Y, Kaneko M, Miyata K, Yatomi Y, Yanagi Y. Bevacizumab and aflibercept activate platelets via FcγRIIa. Invest Ophthalmol Vis Sci. 2015;56(13):8075–8082. doi: 10.1111/j.1538-7836.2008.03212.x.
49. Jedlitschky G, Greinacher A, Kroemer HK. Transporters in human platelets: physiologic function and impact for pharmacotherapy. Blood. 2012;119(15):3394–3402. doi: 10.1182/blood-2011-09-336933.
50. Cox JT, Eliott D, Sobrin L. Inflammatory complications of intravitreal anti-VEGF injections. J Clin Med. 2021;10(5):981. doi: 10.3390/jcm10050981.
51. Baldo BA. Adverse events to monoclonal antibodies used for cancer therapy: focus on hypersensitivity responses. Oncoimmunology. 2013;2(10):e26333. doi: 10.4161/onci.26333.
52. Isabwe GAC, Garcia Neuer M, de Las Vecillas Sanchez L, Lynch DM, Marquis K, Castells M. Hypersensitivity reactions to therapeutic monoclonal antibodies: phenotypes and endotypes. J Allergy Clin Immunol. 2018;142(1):159–170.e2. doi: 10.1016/j.jaci.2018.02.018.
53. Karle AC, Wrobel MB, Koepke S, Gutknecht M, Gottlieb S, Christen B, Rubic-Schneider T, Pruimboom-Brees I, Leber XC, Scharenberg M, et al. Anti-brolucizumab immune response as one prerequisite for rare retinal vasculitis/retinal vascular occlusion adverse events. Sci Transl Med. 2023;15(681):eabq5241. doi: 10.1126/scitranslmed.abq5241.
54. Kwon JW, Jee D. Aqueous humor cytokine levels in patients with diabetic macular edema refractory to anti-VEGF treatment. PLOS One. 2018;13(9):e0203408. Erratum in: PLOS One. 2018;13(11):e0207902. doi: 10.1371/journal.pone.0203408.
55. Maneta E, Aivalioti E, Tual-Chalot S, Emini Veseli B, Gatsiou A, Stamatelopoulos K, Stellos K. Endothelial dysfunction and immunothrombosis in sepsis. Front Immunol. 2023;14:1144229. doi: 10.3389/fimmu.2023.1144229.
56. Rolling CC, Barrett TJ, Berger JS. Platelet–monocyte aggregates: molecular mediators of thromboinflammation. Front Cardiovasc Med. 2023;10:960398. doi: 10.3389/fcvm.2023.960398.
57. Ostrovsky L, King AJ, Bond S, Mitchell D, Lorant DE, Zimmerman GA, Larsen R, Niu XF, Kubes P. A juxtacrine mechanism for neutrophil adhesion on platelets involves platelet-activating factor and a selectin-dependent activation process. Blood. 1998;91(8):3028–3036. doi: 10.1182/blood.V91-8.3028.3028_3028:3036.
58. André P, Hartwell D, Hrachovinová I, Saffaripour S, Wagner DD. Pro-coagulant state resulting from high levels of soluble P-selectin in blood. Proc Natl Acad Sci U S A. 2000;97(25):13835–13840. doi: 10.1073/pnas.250475997.
59. Ivanov II, Apta BHR, Bonna AM, Harper MT. Platelet P-selectin triggers rapid surface exposure of tissue factor in monocytes. Sci Rep. 2019;9(1):13397. doi: 10.1038/s41598-019-49635-7.
60. Suzuki J, Hamada E, Shodai T, Kamoshida G, Kudo S, Itoh S, Koike J, Nagata K, Irimura T, Tsuji T. Cytokine secretion from human monocytes potentiated by P-selectin-mediated cell adhesion. Int Arch Allergy Immunol. 2013;160(2):152–160. doi: 10.1159/000339857.
61. Bakogiannis C, Sachse M, Stamatelopoulos K, Stellos K. Platelet-derived chemokines in inflammation and atherosclerosis. Cytokine. 2019;122:154157. doi: 10.1016/j.cyto.2017.09.013.
62. Matsumoto H, Hoshino J, Mukai R, Nakamura K, Akiyama H. Short-term outcomes of intravitreal brolucizumab for treatment-naïve neovascular age-related macular degeneration with type 1 choroidal neovascularization including polypoidal choroidal vasculopathy. Sci Rep. 2021;11(1):6759. doi: 10.1038/s41598-021-86014-7.
63. Sabetta A, Lombardi L, Stefanini L. Sex differences at the platelet–vascular interface. Intern Emerg Med. 2022;17(5):1267–1276. doi: 10.1007/s11739-022-02994-y.
64. Leng XH, Hong SY, Larrucea S, Zhang W, Li TT, López JA, Bray PF. Platelets of female mice are intrinsically more sensitive to agonists than are platelets of males. Arterioscler Thromb Vasc Biol. 2004;24(2):376–381. doi: 10.1161/01.ATV.0000110445.95304.91.
65. Gremmel T, Kopp CW, Eichelberger B, Koppensteiner R, Panzer S. Sex differences of leukocyte–platelet interactions and on-treatment platelet reactivity in patients with atherosclerosis. Atherosclerosis. 2014;237(2):692–695. doi: 10.1016/j.atherosclerosis.2014.10.095.
66. Kurrelmeyer K, Becker L, Becker D, Yanek L, Goldschmidt-Clermont P, Bray PF. Platelet hyperreactivity in women from families with premature atherosclerosis. J Am Med Womens Assoc (1972). 2003;58(4):272–277.
67. Waissi F, Dekker M, Bank IEM, Korporaal SJA, Urbanus RT, de Borst GJ, Pasterkamp G, Scholtens AM, Grobbee DE, Mosterd A, et al. Sex differences in flow cytometry-based platelet reactivity in stable outpatients suspected of myocardial ischemia. Res Pract Thromb Haemost. 2020;4(5):879–885. doi: 10.1002/rth2.12344.
68. Soo Kim B, Auerbach DS, Sadhra H, Godwin M, Bhandari R, Ling FS, Mohan A, Yule DI, Wagner L, Rich DQ, et al. Sex-specific platelet activation through protease-activated receptors reverses in myocardial infarction. Arterioscler Thromb Vasc Biol. 2021;41(1):390–400. doi: 10.1161/ATVBAHA.120.315033.