

# BROLUCIZUMAB – A NEW PLAYER IN THE FIELD OF ANTI-VEGF THERAPY OF NEOVASCULAR AGE-RELATED MACULAR DEGENERATION. A REVIEW

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*The author declares that no conflict of interests exists in the compilation, theme and subsequent publication of this professional communication, and that it is not supported by any pharmaceuticals company. The study has not been submitted to any other journal or printed elsewhere.*

Received: 29 December 2020

Accepted: 14 March 2021

Available on-line: 20 June 2021



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## SUMMARY

Vascular endothelial growth factor (VEGF) has been identified as a major promoter of the development of choroidal neovascularization in age-related macular degeneration. The development of choroidal neovascularization can be slowed by preventing the binding of vascular endothelial growth factor to cellular VEGF receptor-2 present on vascular endothelial cells, which represents the major proangiogenic stimulus. Advances in the development of anti-VEGF therapy have led to significant improvement in visual acuity outcomes in recent years that neovascular age-related macular degeneration can no longer be considered an incurable disease. Despite its many advantages, the current standard of care, which is the frequent application of VEGF blockers to the vitreous, is a significant burden on both the patient and the healthcare system.

This review is aimed at a new brolocizumab molecule (also known as RTH 258 or formerly ESBA 1008). The article focuses on the molecular aspects of the drug and an overview of the basic preclinical and clinical studies that were performed during drug development. Brolocizumab is a single chain fragment of a humanized monoclonal antibody with a molecular weight of 26 kDa that inhibits VEGF-A. Preclinical animal studies have shown good penetration of the molecule through the retina with minimal systemic exposure. The SEE study (phase 1/2) demonstrated safety and tolerability after drug administration. The OSPREY (phase 2) study demonstrated the same efficacy of brolocizumab on visual acuity in the 8-week dosing regimen compared to aflibercept. In the same study, patients were also pilot tested in a 12-week dosing regimen. The HAWK and HARRIER studies (phase 3) demonstrated the efficacy of the drug at a dose of 6 mg in a 12-week dosing schedule in 55.6 % and 51 % of patients, respectively.

**Key words:** brolocizumab, vascular growth factor, ARMD, CNV, anti-VEGF

Čes. a slov. Oftal., 78, 2022, No. 1, p. 3–8

## INTRODUCTION

Age-related macular degeneration (ARMD) continues to constitute the main cause of blindness in industrially developed countries, with a worldwide prevalence of 8.69% [1]. At present ARMD affects 196 million people on the planet, and by the year 2040 this number is projected to increase to 288 million people [2].

ARMD is a chronic progressive disease, which may develop into two main advanced forms: neovascular ARMD (wet form) or geographic atrophy (dry form) [3]. Despite the fact that neovascular ARMD represents only 20% of all cases, it is responsible for 90 % of cases of loss of sight in all patients diagnosed with ARMD [3]. Neovascular ARMD is characterised by the presence of a cho-

roidal neovascular membrane (CNV), which constitutes pathological angiogenesis leading to the infiltration of fluid. This fluid is accumulated intraretinally, subretinally or beneath the retinal pigment epithelium (RPE). Further changes in neovascular ARMD may include rupture of the RPE, development of hard exudates, haemorrhages or a disciform scar [3,4,5,6,7]. Without therapeutic intervention, the aforementioned clinical abnormalities lead to progressive damage to loss of photoreceptors and subsequent reduction of visual acuity (VA) [8].

### Recent advances in the treatment of neovascular ARMD

The main aim of the treatment of ARMD is the maintenance or improvement of central VA. This goal can be

achieved by the “drying” of the affected retina by halting the growth of newly formed vessels with subsequent reduction of infiltration. The fundamental promoter of the progression of CNV has been identified as vascular endothelial growth factor (VEGF) [3]. Suppression of CNV growth by VEGF blocking has been demonstrated in mice and monkey models of angiogenesis [9,10,11]. The progression of CNV can be slowed by preventing the binding of VEGF to cellular VEGF receptor-2 present on vascular endothelial cells, which is the main pro-angiogenic stimulus [12]. Antibodies preventing the binding of VEGF protein to the receptor (anti-VEGF) reduce the infiltration of fluid from the CNV and lead to the slowing or regression of growth of the CNV [9,13]. In recent years, advances in the development of anti-VEGF therapy have led to a pronounced improvement in the results of VA, with the result that today neovascular ARMD can no longer be considered an incurable disease [13].

The first anti-VEGF preparation to be approved for use by the American Food and Drug Administration (FDA) in 2004 was pegaptamib sodium. This is an aptamer, which selectively blocks the isoform VEGF165 [14]. The next preparation, ranibizumab, is a fragment of an anti-VEGF antibody blocking all isoforms of VEGF-A. Ranibizumab was approved by the FDA in 2006 following the registration trials ANCHOR and MARINA [13,15,16]. The next two anti-VEGF preparations represent recombinant fusion proteins. The first of these was aflibercept, approved in 2011 in the USA and 2012 in Europe on the basis of the registration trials VIEW 1 and 2 [17]. The second fusion protein was the molecule conbercept, approved for use in China in 2013 on the basis of the PHOENIX trial [18]. In addition, there are two preparations that are relatively widely used without the approval of registration authorities, thus in an off-label regimen, namely bevacizumab and ziv-aflibercept [19,20].

With regard to the chronic character of the pathology, the current standard of anti-VEGF therapy for neovascular ARMD requires long-term intravitreal application [21]. However, long-term intravitreal application places a considerable burden on both the patient and the healthcare system. With regard to the above circumstances, it is difficult to convert the results of clinical trials into actual everyday practice, and the results of treatment in “real clinical practice” are worse in comparison with the results produced in trials [22]. During the course of the observation period, due to the aforementioned disadvantages there was a relaxation of the originally fixed therapeutic regimens to allow alternative methods of administration, such as a pro re nata (PRN) regimen or Treat and Extend [23]. The aim of these alternative dosing regimens is greater individualisation of the therapeutic schema for the patient, on the basis of which it is possible to save on both financial resources and the capacities of the healthcare system. In 2019 the new preparation brolocizumab was approved, which demonstrated longer therapeutic efficacy in clinical trials, with an improvement of VA upon application every 12 weeks. This summary article focuses

on the facts concerning the development of brolocizumab, and also presents an overview of the preclinical and clinical trials focusing on its safety and efficacy.

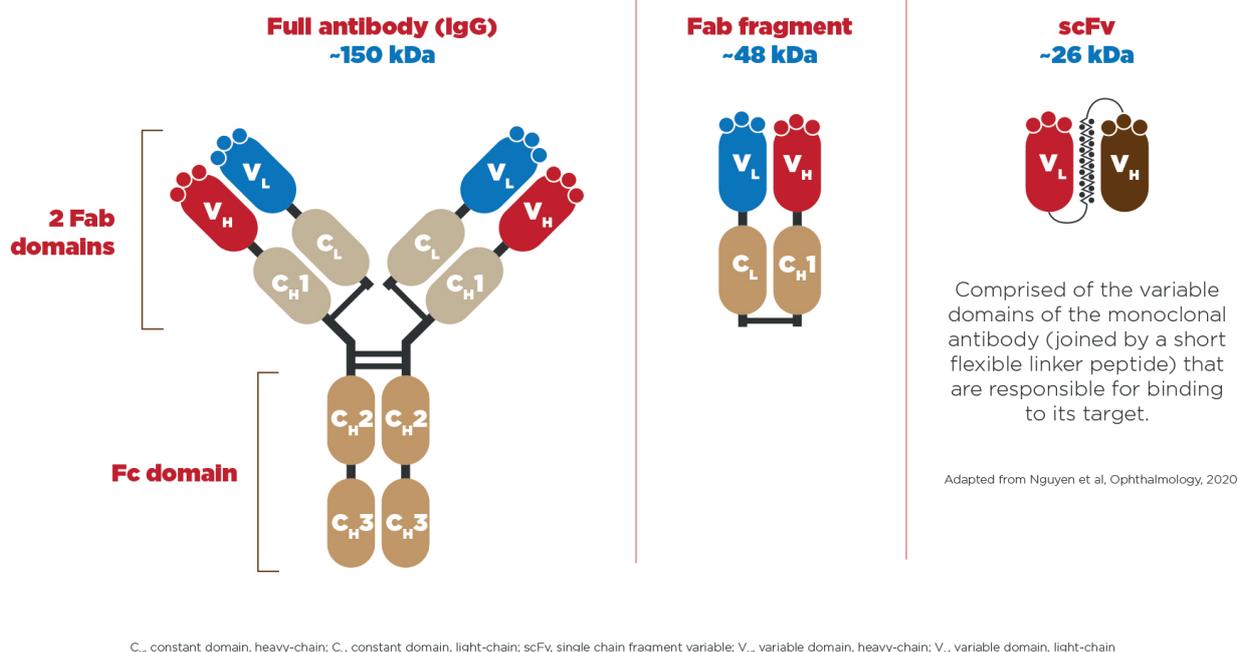
## BROLUCIZUMAB

### Molecular aspects

Brolocizumab (also known as the molecule RTH 258 or formerly ESBA 1008) is a single-chain fragment of a humanised monoclonal antibody (scFv), which inhibits VEGF-A [24]. ScFv is an autonomous binding part of the antibody, stripped of the heavy molecular structure but with fully preserved binding capacity [25,26]. From a pharmacological perspective, single-chain fragments are very attractive molecules. Their small dimension and the absence of crystallising domains gives them a functional advantage upon use in vivo, such as excellent biological accessibility and low immunogenicity [27]. In comparison with full IgG, they have better penetration into tissues, and therefore a corresponding better local effect with a longer duration, furthermore with lesser adverse systemic effects [28,29]. Brolocizumab is produced using recombinant DNA technology, and its resulting molecular weight is 26 kDa, thus the lowest among all the current anti-VEGF preparations [24]. The small dimension of the molecule (aflibercept 97 is as high as 115 kDa and ranibizumab 48 kDa) and its excellent solubility enables the production of a solution with a concentration of as high as 120 mg/ml. Such a high concentration enables the administration of 6 mg of brolocizumab in standard 50µm intravitreal application. Thanks to the high concentration, a larger quantity (number) of brolocizumab molecules is supplied to the vitreous space in standard intravitreal application. As a result, its binding capacity to VEGF-A is 11 to 22 times greater in comparison with aflibercept or ranibizumab, which leads to a prolonging of the therapeutic effect of the drug [24]. (Fig. 1).

### Preclinical trials

The high affinity of brolocizumab to the isoform of VEGF-A, with subsequent blocking of binding to VEGF receptor 1 and 2 has been demonstrated in several in vitro trials [30]. The pharmacokinetic properties of brolocizumab have been studied on a monkey model of the species crab-eating macaque (*Macaca fascicularis*). In the trial, a dose of brolocizumab was applied in a quantity of 1 or 6 mg into both eyes of 9 primates [30]. The trial determined that the concentration of brolocizumab following application of the drug in the central retina reached 42% of the vitreous concentration, and in the choroid 18 % of the vitreous concentration. It was also determined that the average half-life, clearance of the drug from the ocular tissues is 2.4±0.3 days. The maximum serum concentration of the drug was approximately 3500 times lower in comparison with the concentration in the vitreous cavity, and serum clearance was 51.0 hours. On the basis of the results of this trial, it was determined that brolocizumab penetrates into the choroid, but systemic penetration is



C<sub>H</sub>, constant domain, heavy-chain; C<sub>L</sub>, constant domain, light-chain; scFv, single chain fragment variable; V<sub>H</sub>, variable domain, heavy-chain; V<sub>L</sub>, variable domain, light-chain

**Fig. 1.** Schematic comparison of the molecular structure of a single-chain fragment of a humanised monoclonal antibody with a whole IgG antibody and a fragment thereof (ranibizumab). Adopted from Nguyen et al. [30]

minimal. In further trials on macaques, no ocular or systemic toxicity of brolocizumab was determined upon intravitreal application, and only minimal ocular inflammatory manifestations appeared [30]. The pharmacokinetic properties of brolocizumab were also examined in macaques upon intravenous administration in a concentration of 2 mg/kg. The half-life of serum clearance in this trial was determined at 5.6±1.5 hours.

All the preclinical trials demonstrated the potential of brolocizumab in blocking VEGF, with minimal systemic effect and toxicity in the species macaca fascicularis.

## CLINICAL TRIALS WITH BROLUCIZUMAB

### Phase 1/2 SEE trial

The safety and efficacy of the brolocizumab preparation were first assessed in patients with untreated ARMD in the SEE trial [24]. This was a prospective, multicentric, randomised, double-blind trial. In the first phase, the maximum feasible dose (MFD) of brolocizumab was stipulated in a quantity in increasing application from 0.5 mg to 6 mg. In the next phase the effectiveness of the MFD was evaluated in comparison with 0.5 mg ranibizumab. The results confirmed a similar effectiveness of brolocizumab in a dose of 4.5 mg and 6 mg in comparison with 0.5 mg ranibizumab, with reduction of central macular thickness (the difference in the change in comparison with ranibizumab in the 1st month was 22.86 µm in the case of 4.5 mg and 19.40 µm in the case of 6 mg). A difference was also demonstrated in the interval for the requirement for

subsequent treatment, which was 75 days in the group with 6 mg brolocizumab and 45 days in the case of ranibizumab ( $p = 0.04$ ). No unexpected adverse effects were demonstrated in the trial in connection with the application of brolocizumab [24].

### Phase 2 OSPREY trial

Based on the results of the SEE trial, which demonstrated the need for reapplication of brolocizumab with an interval 30 days longer in comparison with ranibizumab, the OSPREY trial was conducted [24,32]. This trial examined the safety and efficacy of the brolocizumab preparation in comparison with aflibercept (approved application every 8 weeks). The study incorporated patients with untreated neovascular ARMD, randomised into two branches in a ratio of 1:1 (aflibercept 2 mg and brolocizumabu 6 mg). The trial was divided into 3 phases. In the first saturation phase between the commencement of the trial and week 12, both substances were applied at an interval of every 4 weeks in both groups. In the second phase the interval of reapplications of both preparations was extended to 8 weeks, and the evaluation of this phase took place in the 40th week of the trial. In the final phase of the trial, the interval was extended to 12 weeks in the branch with brolocizumab, whereas in the aflibercept branch the interval of applications was maintained at 8 weeks with the final evaluation of the results in the 56th week of the trial [32].

The primary aim of the trial was to compare best corrected VA in both groups after 12 and 16 weeks of the trial.

No statistically significant difference was demonstrated in the gain of letters either in the 12th week of the trial measured on an ETDRS chart (5.75 letters in the case of brolocizumab and 6.89 letters for aflibercept). This trend persisted also after the phase of dosing every 8 weeks in the 40th week of the trial (6.25 letters in brolocizumab vs. 5.75 in aflibercept). Furthermore, a higher percentage of patients with complete subsidence of intraretinal fluid (IRF) and subretinal fluid (SRF) was recorded in the group treated by brolocizumab (61% brolocizumab vs. 35% aflibercept). In half of the patients in the phase of dosage of brolocizumab every 12 weeks, best corrected VA remained stable also at the end of the observation period in week 56. No difference in the safety profile of both preparations was recorded in the trial [32].

### Phase 3 HAWK and HARRIER trials

On the basis of the results of the first and second phase trials, a design of a third phase trial was prepared. Within the framework of the trial, the individual dynamics of the activity of the disease were taken into account, such as best corrected VA, therapeutic response to the saturation phase of treatment or anatomic results [32]. Based on the dynamics of the pathology, the patients were divided into a dosing regimen with an interval of applications either of every 12 or every 8 weeks. The HAWK and HARRIER trials were 2-year randomised multicentric trials, in which the effect of aflibercept 2 mg was compared with brolocizumab 3 mg (only in the HAWK trial) and 6 mg upon treatment of neovascular ARMD. The trial began for both preparations with the initial saturation phase, in which the first 3 applications of both preparations were applied at a monthly interval (application at the beginning of the trial and at 4 and 8 weeks). The branch of patients treated with aflibercept then received application of the preparation in the standard approved regimen every 8 weeks. The patients in the branch treated with brolocizumab (3 mg and 6 mg) were subsequently treated at an interval of 12 weeks in the case that no signs of activity of the pathology were present at the follow-up visits (in weeks 16, 20, 32 and 44 of the HAWK trials and additionally in weeks 28 and 40 in the HARRIER trial) [33]. The primary aim of both trials in the 48th week was to determine whether best corrected VA in the patients treated with brolocizumab was worse in comparison with those treated with aflibercept. The secondary aim was to determine the proportion of patients treated with brolocizumab suitable for application within a 12-week interval, the anatomical results (presence of SRF/IRF) and the safety profile of the preparation.

The results demonstrated comparable effectiveness on best corrected VA in the case of both preparations. In the HAWK trial, the average gain of ETDRS letters in the group treated with brolocizumab 3 mg was +6.1 letters, in the group with brolocizumab 6 mg +6.6 letters and in the group with aflibercept 2 mg +6.8 letters of ETDRS chart. Similar results were attained in the HARRIER trial, where the average gain was +6.9 letters in patients treated with brolocizumab 6

mg and +7.6 letters in the patients treated with aflibercept 2 mg. Central macular thickness (CMT) was reduced significantly in the patients treated with brolocizumab at a follow-up examination in the 16th week of the trial, and a significant difference was maintained throughout the entire observation period up to the 48th week of the trial. In the patients treated with brolocizumab, in all cases there was a significantly lower proportion of patients with persistent SRF and IRF. In the 48th week of the trial, 55.6% of the patients treated with brolocizumab 6 mg in the HAWK trial and 51.0% of the patients in the HARRIER trial did not manifest any signs of activity of the disease at an interval of applications of 12 weeks. Adverse effects were comparable with the phase 2 trials. The most frequently recorded were conjunctival suffusion, pain in the eyes and vitreous turbidities in less than 5% of cases. Special attention was focused on the presence of uveitis. This adverse effect was recorded in 2.2% of cases in the branch treated with brolocizumab 6 mg in the HAWK trial and in 0.8% of cases in the HARRIER trial. Analogous values for the group treated with aflibercept were 0.3% in the HAWK trial and 0% in the HARRIER trial. Recorded cases of uveitis were evaluated as mild in 90% of cases and resolved with local application of corticoid therapy, curing the condition without long-term consequences [33]. The 2-year results of the HAWK and HARRIER trials were published recently [34]. The results of best corrected VA show a comparable gain of letters in both trials in the case of both preparations after 96 weeks of observation and are within a range from 5.3 to 6.6 letters of ETDRS. The resulting gain in both groups is lower in comparison with the previous study of anti-VEGF therapy [34,35,36]. This probably concerns a ceiling effect, because on the basis of the entry criteria, recruitment of patients was permitted if they had best corrected VA worse or equal to 78 EDTRS letters, and the average baseline best corrected VA in both trials was 61 letters. This value is approximately 8 letters better than the previous trial, in which average best corrected VA was 53 letters of ETDRS chart [35,36]. Over the course of 96 weeks of observation, a significantly larger reduction of CMT is recorded already in the 48th week of the trial. In connection with this finding, in patients treated with brolocizumab there also remains a significantly lower proportion of patients with persistent SRF and IRF. This result indicates the greater potential of brolocizumab in reducing vascular infiltration. Such results are probably due to the uniqueness of the molecule and the possibility of transporting a larger quantity of effective molecules into the affected region, as has been described within the framework of preclinical trials [24,30]. Although to date no study has demonstrated a direct correlation between the degree of reduction of CMT and the gain of ETDRS letters, reduction of CMT measured on optical coherence tomography (OCT) is considered the best parameter for evaluating therapeutic success in the clinical medical recommendations [11,14,37]. In the 96th week, 45.4% of patients treated with brolocizumab 6 mg in the HAWK trial and 38.6% of patients in the HARRIER trial had undergone the entire observation period of the study with an interval of applications of 12 weeks. In the group of patients who did not manifest signs of activity

after the first year of observation, in the individual trials their proportion was 81.5% and 75.6% respectively. Furthermore, the design of the trial did not enable the conversion of patients who had been administered application of brolicizumab in an interval of every 8 weeks back to a 12-week interval also upon improvement of the parameters, and so it is possible to expect that there was a certain underestimation in comparison with real practice. The safety profile of the drug, from the perspective of both local and general adverse effects, is on a good level in comparison with other anti-VEGF preparations.

## SUMMARY OF CLINICAL IMPLICATIONS ENSUING FROM THE TRIALS

At present, VEGF blocking represents the gold standard in the therapy of neovascular ARMD. Despite the unequivocal improvement in the results of application of this therapy, it continues to place a considerable burden on the healthcare system, both financially and in terms of healthcare staff. For

this reason, it remains a persistent challenge to maintain the effectiveness of treatment while reducing the number of necessary visits. The result of this endeavour has been the development of alternative dosing regimens such as PRN or Treat and Extend. However, the results of recent studies indicate better results in fixed dosing regimens in comparison with other alternatives [38].

According to the trial, in 35–50% of patients the brolicizumab molecule enables application in a fixed regimen with an interval of 12 weeks. This is the largest interval between all the anti-VEGF preparations (for aflibercept the interval is 8 weeks, ranibizumab 4 weeks) approved to date by the regulatory authorities. Furthermore, it ensues from the trial that brolicizumab is the most effective preparation in the reduction of IRF and SRF. On the basis of these results, it is possible to consider brolicizumab to be the potential drug of first choice in the treatment of neovascular ARMD in the forthcoming period, even though it is necessary to examine the optimal therapeutic regimen in further studies and in real clinical practice.

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