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Relationship between Aflibercept Efficacy and Genetic Variants of Genes Associated with Neovascular Age-Related Macular Degeneration: The BIOIMAGE Trial

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Keywords
Neovascular age-related macular degeneration · Aflibercept · Vascular endothelial growth factor · VEGF pathway

Abstract
Purpose: To identify the genetic variants of the vascular endothelial growth factor (VEGF) pathway genes and other genes associated with neovascular age-related macular degeneration (nAMD) as possible predictive biomarkers of a favorable treatment response to aflibercept. ***Design:*** A 52-week (with extension phase: 104-week), prospective, open-label, single-arm, multicenter, phase IV trial was conducted in Spain. ***Participants:*** Patients with nAMD were enrolled. ***Methods:*** Aflibercept was administered every 8 weeks until week 48 (after 1-monthly loading doses over 3 months). After week 48, the interval between visits for aflibercept administration was extended by 2 weeks per visit to a maximum of 12 weeks if no evidence of disease activity was observed. A total of 338 SNPs in 90 genes associated with nAMD were analyzed. ***Main Outcome Measures:*** Efficacy was evaluated mainly with best-corrected visual acuity (BCVA), and adverse events (AEs) were reported. Treatment efficacy was defined as an increase in BCVA ≥15 letters versus the baseline visit. Univariate and multivariate logistic regressions were used to associate single-nucleotide polymorphisms (SNPs) and treatment efficacy. ***Results:*** 194 nonconsecutive patients were enrolled, 170 completed the 52-week follow-up, and of the 85 patients who started the extension phase, 77 completed this phase. Mean BCVA increased from baseline to weeks 52 and 104 by 9 and 10 letters ($p = 0.0001$ for both), respectively. The percentages of patients gaining ≥15 letters in weeks 52 and 104 were 33 and 31%, respectively. Multivariate logistic regression showed significant associations of 6 SNPs (in 6 genes) with treatment efficacy: rs12366035 (*VEGFB*; TT; odds ratio [OR] 217), rs25681 (*C5*; AA/AG; OR 19.7/8.3), rs17793056 (*CX3CR1*; CT/CC; OR 8.1/6.2), rs1800775 (*CETP*; CC; OR 6.6), rs2069845 (*IL6*; GG/AA; OR 5.6/3.3), and rs13900 (*CCL2*; CT; OR 4.0). One percent of the patients reported arteriothrombolic events related to aflibercept (cerebrovascular accident) according to the Antiplatelet Trialist Collaboration, and 2% reported serious ocular (retinal pigment epithelial tear, retinal tear, and endophthalmitis) and systemic (cardiac failure, hypersensitivity, and transient ischemic attack) AEs related to

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aflibercept. ***Conclusions:*** Results suggest strong pharmacogenetic associations between one genetic variant of *VEGFB* (TT, rs12366035) and *C5* (AA, rs12366035) genes and the BCVA response after 52-week aflibercept treatment in patients with nAMD. Likewise, the results support the efficacy of aflibercept observed in phase III studies and a good safety profile.

Introduction

The standard treatment for neovascular age-related macular degeneration (nAMD) is intravitreal (IVT) administration of antiangiogenics, for example, vascular endothelial growth factor (VEGF) inhibitors [1, 2]. There are insufficient data at present to determine why treatment response varies despite uniform treatment of all patients. It is known that nAMD is a multifactorial disease influenced by several factors as either causes or proxies, such as age, environment, choroidal neovascularization (CNV) lesion characteristics at baseline, and genetic variations [3–5]. However, the studies trying to unveil the impact of these factors on treatment response are still somewhat limited. Regarding the genetic variants that might be associated with anti-VEGF treatment response, study results are mixed. For instance, some studies found no or only one association in univariate analyses [6–8] between the single-nucleotide polymorphisms (SNPs) of *VEGF-A* and best-corrected visual acuity (BCVA), while other studies found significant associations [9–11], specifically in the SNPs rs699947, rs1413711, and rs3025000. Another example is the *complement factor (CFH)* gene (specifically the SNP rs1061170), as some studies reported an association with BCVA outcomes [9, 12] while others failed to identify any association with treatment [7, 13].

Most of these genetic studies involved the anti-VEGF treatments bevacizumab and ranibizumab. Studies analyzing the association between genetic variants and aflibercept (Eylea; Regeneron Pharmaceuticals) are scarce. Although aflibercept is known as an anti-VEGF treatment, it has a different mechanism of action from bevacizumab and ranibizumab, and the conclusion drawn from these treatments might not apply to aflibercept [14, 15]. Thus, the aim of this study was to identify the genetic variants of the VEGF pathway genes as well as other genes associated with nAMD as possible predictive biomarkers of a good treatment response to aflibercept.

Methods

Trial Design

This phase-IV, prospective, open-label, single-arm, multicenter trial was conducted at 24 trial sites (tertiary health care facilities) in Spain (BIOIMAGE Trial [IMO-AFLI-2013-01], EudraCT No. 2013-002124-17).

Study Population

The main inclusion criteria were: willingness to provide written informed consent; age ≥50 years; a diagnosis of active primary subfoveal CNV (secondary to the AMD) including juxtafoveal lesions affecting the fovea (confirmed through angiography); a baseline BCVA score between 73 and 25 ETDRS (Early Treatment Diabetic Retinopathy Study) letters (inclusive) measured using ETDRS chart at 4 m (Snellen equivalent: 20/40 to 20/320) in the study eye.

The main exclusion criteria (in the study eye) were: prior anti-VEGF treatment, prior or concomitant ocular or systemic treatment (whether experimental or approved) for nAMD; inflammation/infection in or around the eye; prior ocular surgery (including cataract surgery) within 3 months prior to the first aflibercept administration; vascular diseases of the retina; prior glaucoma surgery or vitrectomy; other eye diseases that could cause impaired visual acuity (VA); or inability to undergo a fluorescein angiography or eye fundus photography.

If patients with bilateral disease met the eligibility criteria for both eyes, the eye with the best VA was chosen as the study eye. If the VA was identical for both eyes, the study eye was chosen at the investigator's discretion. The contralateral eye was treated independently with anti-VEGF IVT injections according to routine practice.

Treatment

Patients were treated with aflibercept 2 mg (40 mg/mL) IVT during a 52-week study period, receiving 1 IVT injection once monthly for the first 3 months (loading phase: weeks 0, 4, and 8), and then 1 IVT injection every 8 weeks (q8) until week 48 (scheduled treatment). All patients concluded the study period at week 52. At week 48, patients were invited to participate in a 52-week extension phase. Around half of the patients could not be invited to this phase for administrative/logistic reasons (e.g., patients had already completed the study or trial sites declined to participate in the extension phase, etc.). In the extension phase, the interval between visits for aflibercept administration was extended by 2 weeks per visit (in relation to the period since the last visit) to a maximum of 12 weeks if no evidence of disease activity was observed (treat-and-extend regimen). If there were signs of activity, patients were retreated, and the next visit was reduced by 2 weeks, with a minimum of 8 weeks between visits. Patients concluded the extension phase after 104 weeks.

Trial Procedures

At screening, eligibility criteria, demographic data, medical history, physical examination, concomitant medication, pregnancy test (if applicable), intraocular pressure, indirect ophthalmoscopy, BCVA score, spectral-domain optical coherence tomography (SD-OCT), and adverse events (AEs) were assessed or reported. During all the study visits, concomitant medication, intraocular pressure, indirect ophthalmoscopy, BCVA score, SD-OCT, and AEs were as-

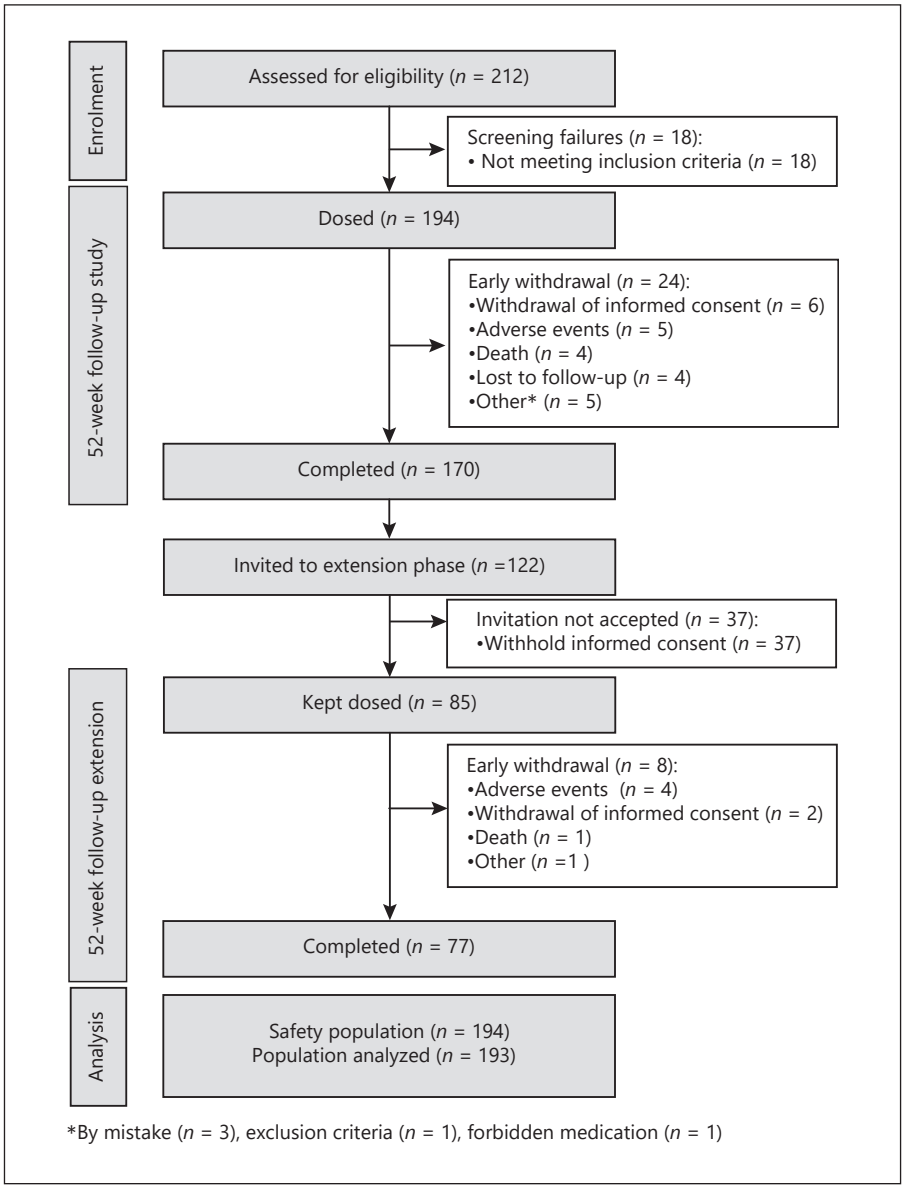

Fig. 1. Flow diagram of the progress through the enrolment, follow-up, and data analysis.

sessed or reported. For the extension phase, patients provided a new written informed consent in week 48, and BCVA score, SD-OCT, and AEs were assessed or reported in all the visits.

Genotyping

Approximately 10 mL of peripheral blood were collected at baseline from each patient. DNA was extracted and purified from leukocytes with the KingFisher Blood DNA kit using the KingFisher Duo equipment (Thermo Scientific). A total of 338 SNPs located in 90 genes associated with nAMD, selected manually according to bibliography, were analyzed (online suppl. Table 1; see www.karger.com/doi/10.1159/000508902 for all online suppl. material). On average 3–5 SNPs for each gene were analyzed using a TaqMan® OpenArray® Genotyping assay (ThermoFisher Scientific). SNPs were selected from public and private human polymorphism databases, prioritizing (whenever possible) the location in gene coding regions, a minor allele frequency >0.3, and a previous association with nAMD. SNPs were genotyped with a QuantStudio 12K Flex real-time PCR system (ThermoFisher Scientific), following the manufacturer's instructions, protocol, and software.

Trial Outcomes

The primary outcome of the study was the determination of the genetic variants of genes associated with nAMD and their influence on aflibercept treatment efficacy (percentage of patients gaining ≥15 ETDRS letters in week 52). Patients were classified according to their response to aflibercept treatment on BCVA measured by ETDRS, as percentage of patients gaining ≥15 ETDRS letters, patients losing <15 ETDRS letters, or patients losing ≥15 ETDRS letters.

Secondary outcomes of the study included: mean change in BCVA, central retinal thickness, and a CNV ratio from baseline to

Table 1. Characteristics of the study population (n = 193)

Demographic data	
Mean age (SD), years	78.5 (7.7)
Median (range)	79.8 (55.9–96.4)
Females:males, *n* (%)	106 (54.9):87 (45.1)
Study eye	
Right:left, *n* (%)	94 (48.7): 99 (51.3)
Mean time from nAMD diagnosis (SD), months	2.8 (13.3)
Median (range)	0.07 (0.03–102.1)
Mean intraocular pressure at baseline (SD), mm Hg	15.6 (2.8)
Median (range)	16.0 (8.0–24.0)
Mean BCVA (SD) at baseline, ETDRS letters	54.2 (13.9)
Median (range)	56.0 (2.0–80.0)
Contralateral eye diagnosed with AMD (yes:no), *n* (%)	102 (52.9): 91 (47.2)
Mean BCVA (SD) under aflibercept treatment, ETDRS letters	
At 24 weeks (n = 186)	62.6 (15.3)
Median (range)	66.0 (17.0–91.0)
At 52 weeks (n = 169)	64.0 (16.0)
Median (range)	68.0 (12.0–90.0)
At 104 weeks (n = 78)	64.3 (16.1)
Median (range)	69.0 (12.0–85.0)

BCVA, best-corrected visual acuity; ETDRS, Early Treatment Diabetic Retinopathy Study; nAMD, neovascular age-related macular degeneration; SD, standard deviation.

week-52 and -104 visits; proportion of patients gaining ≥15 letters; proportion of patients losing <15 letters; proportion of patients with dry retina (no serous retinal detachment and no intraretinal cysts); and time to achieve dry retina. Safety outcomes included ocular and systemic (nonocular) AEs and serious AEs (SAEs). Key AEs were considered to be those defined by the Antiplatelet Trialist Collaboration as arteriothrombolic events (APTC ATE) [16].

Sample Size and Statistical Analysis

This was an exploratory study, and 176 patients were estimated to have a minimum of 30% of patients gaining ≥15 letters in week 52 in order to detect a 20% difference in the proportion of patients gaining ≥15 letters between genotypes, with an 80% power and a 95% confidence interval (CI). Estimating a 10% loss to follow-up, a sample size of 194 patients was planned.

The population analyzed, used for all efficacy analyses, included all patients enrolled in the trial who had received at least 1 dose of aflibercept and who had undergone at least 1 BCVA assessment after baseline. The safety population, used for all safety analyses, included all patients who had received at least 1 dose of the trial drug.

All data were descriptively analyzed. Continuous variables are presented as means and corresponding 95% CIs, medians, SDs, and ranges (minimum to maximum), while categorical variables are shown as frequencies and percentages. Differences between categorical variables were analyzed by χ^2 or Fisher exact tests, as applicable. For continuous variables, changes from baseline were analyzed using the Student t test for paired data. Univariate logistic regressions were used to study the association between each SNP and patients gaining ≥15 letters (yes/no), and odds ratios (OR) and 95% CIs were estimated. Significant SNPs and significant genotypes in univariate analysis were included in a multivariate logistic regression model. SNPs with >25 missing data were not considered. The level of significance used for all tests was 0.05. No imputation for missing data was performed.

Data analysis was performed using the SAS® statistical package for Windows (version 9.4; SAS Institute Inc., Cary, NC, USA).

Results

Patient Disposition, Baseline Characteristics, and Exposure

A total of 194 nonconsecutive patients were enrolled in the trial between December 16, 2013, and March 30, 2017, and 170 of them (87.6%) completed the 52-week follow-up study (Fig. 1). A total of 122 patients from the 170 who completed the 52-week study were invited to participate in the 52-week extension phase; 85 patients (43.8%) agreed to participate, and 77 (39.7%) completed this phase. All 194 patients were included in the safety populations, and 193 (99.5%) were analyzed.

Baseline characteristics are shown in Table 1. Briefly, mean ± SD age at trial inclusion was 78.5 ± 7.7 years, 106 (54.9%) were female, all were Caucasian, and the time from nAMD diagnosis to trial inclusion was 2.8 ± 13.3 months. Around half of the patients (n = 102; 52.9%) had AMD diagnosis in the contralateral eye, and of these, 57 (55.9%) had nAMD (33 of them [57.9%] also in treatment).

Table 2. Multivariable logistic regression analysis of SNPs associated with at least 15 ETDRS letters gained in BCVA

Gene (SNP)	Genotypes	Odds ratio (95% CI)	*p* value
At week 52[a] (n = 170)			
VEGFB (rs12366035)	CT (*n* = 62) vs. CC (*n* = 66)	2.50 (0.89–7.00)	0.0806
	TT (*n* = 8) vs. CC (*n* = 66)	216.92 (11.67–4,033.9)	0.0003
CX3CR1 (rs17793056)	CC (*n* = 31) vs. TT (*n* = 39)	6.18 (1.34–28.57)	0.0197
	CT (*n* = 66) vs. TT (*n* = 39)	8.10 (2.08–31.61)	0.0026
CETP (rs1800775)	AA (*n* = 41) vs. AC (*n* = 46)	1.39 (0.37–5.16)	0.6246
	CC (*n* = 49) vs. AC (*n* = 46)	6.62 (1.99–22.00)	0.0020
IL6 (rs2069845)	AA (*n* = 53) vs. AG (*n* = 61)	3.31 (1.13–9.67)	0.0291
	GG (*n* = 22) vs. AG (*n* = 61)	5.63 (1.39–22.73)	0.0153
C5 (rs25681)	AA (*n* = 33) vs. GG (*n* = 33)	19.73 (3.59–108.42)	0.0006
	AG (*n* = 70) vs. GG (*n* = 33)	8.28 (1.74–39.35)	0.0078
CCL2 (rs13900)	CT (*n* = 57) vs. CC (*n* = 73)	3.99 (1.42–11.22)	0.0088
	TT (*n* = 6) vs. CC (*n* = 73)	2.01 (0.25–16.00)	0.5101

BCVA, best-corrected visual acuity; CI, confidence interval; ETDRS, Early Treatment Diabetic Retinopathy Study; SNP, single-nucleotide polymorphism. [a] Number of observations: 136.

Visual Acuity

The mean ± SD BCVA score increased from 54.2 ± 13.9 ETDRS letters at baseline to 64.0 ± 16.0 ETDRS letters in week 52, and then remained constant (64.3 ± 16.1) until week 104 (Table 1). This corresponded to mean (95% CI) increases of 9.1 (6.9–11.2) and 10.1 (6.8–13.3) ETDRS letters ($p = 0.0001$ for both), respectively. The BCVA from week 48 to week 104 remained constant in the extension phase: mean (95% CI) change of 0.13 letters (–1.65 to 1.90). The proportions of patients gaining ≥15 letters and patients not losing ≥15 letters in week 52 were 33.0% ($n = 57$) and 93.1% ($n = 161$), respectively, but 30.9% ($n = 25$) and 92.6% ($n = 75$) in week 104, respectively.

Multivariate logistic regression revealed a significant effect of 6 SNPs (in 6 genes) on gaining ≥15 letters in BCVA in week 52 (Table 2). Thus, the odds of gaining ≥15 letters in BCVA after 52 weeks of aflibercept treatment were higher in patients with genotypes TT in rs12366035 (*VEGFB*; OR 216.9; $p < 0.001$), AA/AG in rs25681 (*C5*; OR 19.7/8.3; $p < 0.001/0.01$), CT/CC in rs17793056 (*CX3CR1*; OR 8.1/6.2; $p < 0.01/0.05$), CC in rs1800775 (*CETP*; OR 6.6; $p < 0.01$), GG/AA in rs2069845 (*IL6*; OR 5.6/3.3; $p < 0.05/0.05$), and CT in rs13900 (*CCL2*; OR 4.0; $p < 0.01$). The proportion of patients gaining ≥15 letters in BCVA after 52 weeks of aflibercept treatment according to the genotype of the 6 SNPs obtained from the multivariate logistic regression is shown in online supplementary Table 2.

Key Anatomic Measures

The mean ± SD central retinal thickness decreased from 371.4 ± 119.1 μm at baseline to 236.7 ± 47.6 μm in week 52 and to 245.8 ± 57.3 μm in week 104. This corresponded to a mean (95% CI) reduction of 131.9 (147.8–116.0) and 128.9 (152.1–105.7) μm ($p < 0.0001$ for both), respectively. The mean ± SD CNV ratio decreased from baseline (1.37 ± 1.16 disk areas) to week 52 (1.01 ± 1.11 disk areas) by 0.28 (95% CI 0.45–0.12) disk areas ($p = 0.0010$), and to week 104 (0.87 ± 0.94 disk areas) by 0.26 (95% CI 0.51–0.01) disk areas ($p = 0.0412$).

Pathology Hallmarks

There was a decrease from baseline to weeks 52 and 104 in the proportion of patients with macular edema (by around 76 and 75%, respectively), serous retinal detachment (by around 80 and 76%, respectively), intraretinal cysts (by around 84 and 87%, respectively), and hemorrhages (by around 95 and 97%, respectively; Fig. 2). The proportion of patients with dry retina increased from 14.0% ($n = 27$) at baseline to 78.0% ($n = 135$) in week 52 and to 75.3% ($n = 61$) in week 104. In addition, the mean (95% CI) time to achieve dry retina was 9.5 (7.6–11.4) weeks.

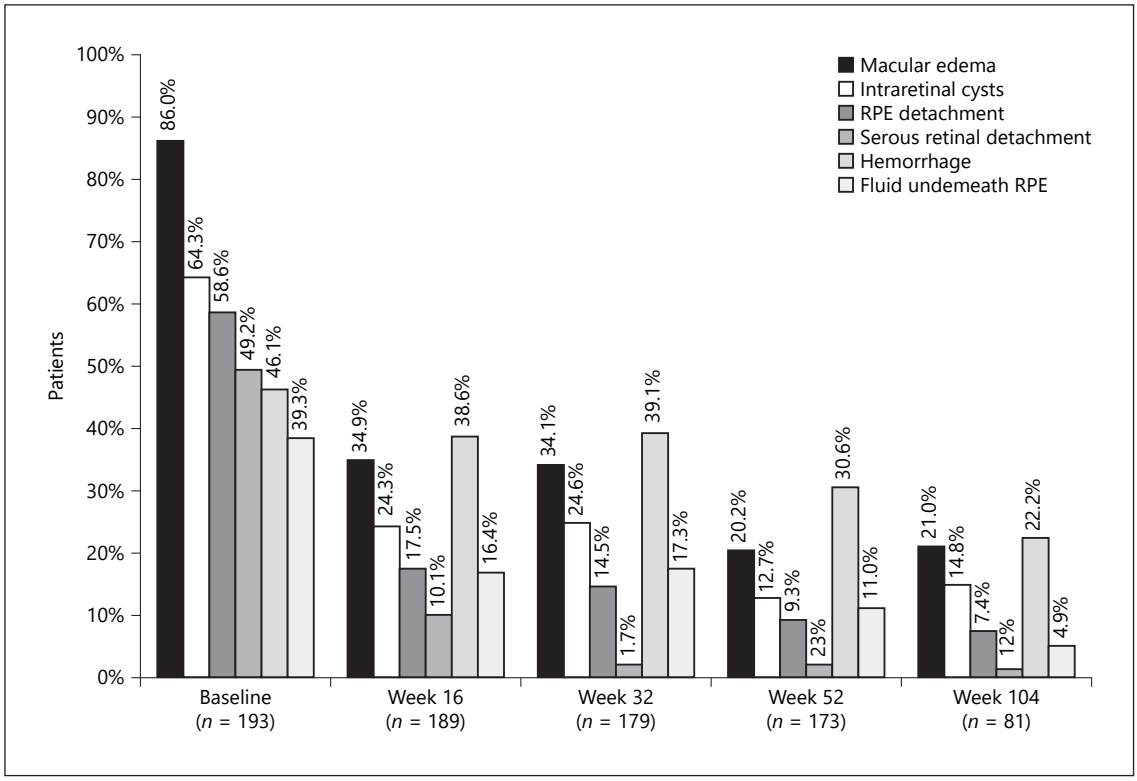

Fig. 2. Patients with serious retinal detachment, intraretinal cysts, macular edema, hemorrhages, fluid underneath of the retinal pigment epithelium (RPE), and RPE detachment over time.

Exposure to Aflibercept

The proportion of patients included in the extension phase that reached an interval between visits of 12 weeks was 42.9% ($n = 36$), while the median number of injections in this phase was 4 (interquartile range: 4–5; mean ± SD 4.5 ± 0.9).The most frequent numbers of injections were 4 (52.4%; $n = 44$) and 5 (22.6%; $n = 19$), and the maximum number of injections was 6 (19.1%; $n = 16$). Finally, the median interval between visits during the extension phase was 11.0 (interquartile range: 9.0–12.1) weeks (mean ± SD 10.6 ± 1.6).

Safety

A total of 334 AEs occurred during the 104-week treatment period: 91 ocular (in 57 [29.4%] patients) and 243 systemic AEs (in 111 [57.2%] patients). There were 5 (2.6%) fatalities during the trial (1 case each, 0.5%): myocardial infarction, sudden death, colon cancer, colon cancer metastasis, and pancreatic neoplasm (Table 3). No death was considered to be related to the trial treatment. Two patients (1.0%) reported AEs considered APTC ATEs related to trial treatment (cerebrovascular accident in both), 4 patients (2.1%) ocular SAEs related to trial treatment (retinal pigment epithelial tear in 2 patients, retinal tear in 1 patient, and endophthalmitis in another patient), and 3 patients (1.6%) systemic SAEs related to trial treatment (1 case of each: cardiac failure, hypersensitivity, and transient ischemic attack). A total of 53 patients (27.3%) reported nonserious ocular AEs, with 11 of them experiencing ocular AEs considered related to trial treatment. No patient reported nonserious systemic AEs related to trial treatment.

Discussion

The BIOIMAGE trial evaluated the association of 338 SNPs (326 SNPs genotyped) with a gain of at least 15 letters in BCVA after 1 year of aflibercept treatment. In 6 SNPs of 6 genes, 9 genotypes were identified as independent genetic variations that might explain the good response to aflibercept after 1 year of treatment. Strong associations were found for the TT genotype of rs12366035 in the *VEGFB* gene and the AA genotype of rs25681 in the

Table 3. Summary of adverse events

	Safety population (n = 194), n (%)
Serious adverse events	
Fatal APTC ATE events (not related to trial treatment)	2 (1.03)
Myocardial infarction	1 (0.52)
Sudden death	1 (0.52)
Deaths (not related to trial treatment)	3 (1.55)
Colon cancer	2 (1.03)
Pancreatic neoplasm	1 (0.52)
Nonfatal APTC ATE events	5 (2.58)
Related to trial treatment:	
Cerebrovascular accident	2 (1.03)
Nonrelated to trial treatment:	
Myocardial infarction	2 (1.03)
Ischemic stroke	1 (0.52)
Other nonfatal serious adverse events	
Ocular	6 (3.09)
Related to trial treatment	
Retinal pigment epithelial tear	2 (1.03)
Retinal tear	1 (0.52)
Endophthalmitis	1 (0.52)
Nonrelated to trial treatment	
Endophthalmitis	1 (0.52)
Peripheral ischemia	1 (0.52)
Systemic (nonocular)	25 (12.89)
Related to trial treatment	
Cardiac failure	1 (0.52)
Hypersensitivity	1 (0.52)
Transient ischemic attack	1 (0.52)
Nonrelated to trial treatment	22 (11.34)
Adverse events (AEs)	
Nonserious ocular AEs	53 (27.32)
Related to trial treatment:	11 (5.67)
Selected AEs (experienced by ≥2 patients)	
Conjunctival hemorrhage	3 (1.55)
Nonrelated to trial treatment:	45 (23.20)
Selected AEs (experienced by ≥5 patients)	
Age-related macular degeneration[a]	13 (6.70)
Conjunctivitis	8 (4.12)
Intraocular pressure increase	6 (3.09)
Nonserious systemic (nonocular) AEs (not related to trial treatment)[b]	93 (47.94)

APTC ATE: Antiplatelet Trialists' Collaboration arteriothrombolic event.

[a] Eleven patients reported this AE in the contralateral eye and 2 in the study eye (nonrelated to trial treatment). [b] The most frequently reported were nasopharyngitis (n = 13, 6.7%), back pain (n = 9, 4.6%), urinary tract infection (n = 6, 3.1%), upper respiratory tract infection (n = 5, 2.6%), and vertigo (n = 5, 2.6%).

C5 gene. To our knowledge, BIOIMAGE is the first study to assess the genetic variants of genes associated with nAMD as predictive biomarkers of aflibercept treatment response. The only study that has investigated the effect of genetic factors on aflibercept was conducted by Kawashima et al. [17]. The authors only assessed *CFH* (rs1061170 and rs800292) and *ARMS2* (rs10490924) genes as predictors of treatment response in AMD and polypoidal choroidal vasculopathy patients refractory to ranibizumab, switching to aflibercept. No association between gene genotype and treatment response was observed by the authors.

Regarding the genotypes of the SNPs of *VEGFB* and *C5* genes, results showed that patients with TT genotype in rs12366035 (vs. CC) and AA genotype in rs25681 (vs. GG) had higher odds of gaining at least 15 letters in BCVA. It is important to note that these odds were accompanied by very wide 95% CIs, probably due to the low number of patients (in TT genotype) and the high variability in treatment response (in both genotypes). Although associations between AMD and the genetic variants of both *VEGFB* and *C5* genes have been studied before, this is the first time that genetic variants of these genes seem to be related with the responses to anti-VEGF treatment. Baas et al. [18] who studied the association between the *C5* gene variants and AMD found that the heterozygote allele of rs25681 had lower risks for late AMD than the other genotypes. However, these findings could not be confirmed consistently in 3 replication populations. In the case of the *VEGFB* gene variants, a study that included rs12366035 suggested that no genetic variant was associated with nAMD [19]. Thus, more studies are needed in order to draw more firm conclusions about the importance of these genotypes, in particular the TT genotype in rs12366035 as its prevalence seems to below. Interestingly, genetic variants commonly cited as potential pharmacogenetic markers of the nAMD treatment did not show an association with aflibercept, e.g., rs699947 and rs3025000 (both in *VEGFA*), rs7993418 (*VEGFR*), rs1136287 (*SERPINF1*), and rs6828477 (*VEGFR2*) [11, 20–22].

Regarding the efficacy, the results in the BIOIMAGE trial were generally similar to those reported in the phase III trials VIEW 1 and VIEW 2, 2 similarly designed randomized, double-masked, active-controlled, parallel-group, 96-week studies [23, 24]. One of the groups received for the first 52 weeks aflibercept (2 mg q8) after 3 initial monthly injections, then patients received injections at least every 12 weeks, with monthly evaluations for interim injections based on prespecified retreatment criteria (capped PRN regimen) until week 96. Although BIOIMAGE and VIEW 1/VIEW 2 regimens were not exactly the same in the second year, results were similar at the 52- and 96-week follow-ups (BIOIMAGE vs. VIEW 1 and VIEW 2 jointly) [23]: BCVA increase (9 vs. 8 and 10 vs. 8 ETDRS letters, respectively), proportion of patients gaining ≥15 letters (33 vs. 31 and 31 vs. 33%, respectively), patients losing <15 letters (93 vs. 95 and 93 vs. 92%, respectively), decrease in central retinal thickness (132 vs. 139 and 129 vs. 133 μm, respectively), and proportion of patients with dry retina (78 vs. 68 and 75 vs. 50%, respectively). The differences in patients with dry retina might be due to the way of measuring the persistent fluid in the eye as SD-OCT was used in the BIOIMAGE trial while time-domain OCT was used in the VIEW 1/VIEW 2 trials. Therefore, the visual and anatomic outcomes achieved by aflibercept in the BIOIMAGE trial, which included a scheduled regimen during the 1st and 2nd years, were similar to those observed in aflibercept pivotal trials.

In clinical practice, the treat-and-extend regimen is gaining popularity. With this treatment approach, the patient receives treatment at every visit; however, the interval between visits is gradually increased once stabilization of the disease is achieved. If the patient worsens, the treatment interval decreases accordingly. During the 2nd year of the BIOIMAGE trial, a treat-and-extend regimen followed with at least 4 of 10 patients reaching an interval between visits of 12 weeks and a mean number of injections <5. These results are in line with studies focused on a treat-and-extend regimen like the ALTAIR study [25], which compares different treat-and-extend regimens (2- vs. 4-week treatment intervals) with aflibercept. The BIOIMAGE results of the second year are important as they show the efficacy of aflibercept treatment in a treat-and-extend regimen, stressing the load of support work on health care facilities. In addition, the results in the treat-and-extend regimen of the BIOIMAGE trial, particularly the amount of patients reaching an interval of 12 weeks between visits, are associated with the methodology reported in clinical trials of new molecules such as brolucizumab [26] and abiciparpegol (available in clinical trials: NCT02462486 and NCT02462928), whose studies are based on 12-week interval maintenance IVT injections since the beginning.

With regard to the safety and tolerability of aflibercept, all the AEs reported were consistent with the safety profile seen during clinical development [23] and listed in the Summary of Product Characteristics [27]. It is important to mention that the proportion of patients with conjunctival hemorrhage was lower in the BIOIMAGE trial than in the Summary of Product Characteristics: 25 vs. 3% (combining both nonrelated and related AEs). This rate is consistent with the result in the ALTAIR study, where conjunctival hemorrhage rates were 2.4 and 5.7% for the 2- and 4-week treatment intervals regimens, respectively [28].

There are some limitations in this trial; the most relevant was the lower-than-expected number of patients included in the genetic variant analysis at the 52-week follow-up, which reduced the statistical power from 80 to 71.5% (n = 136). Another limitation was the single-arm design that did not allow the proper comparison of the

efficacy outcomes (VA, key anatomic measures, and pathology hallmarks). Therefore, the efficacy results are only compared with data from studies with a different treatment administration schedule. However, the latter limitation is due to the design of the study focused in the primary outcome, which is of significance as this is the first study assessing genetic variants as predictive biomarkers of aflibercept treatment response.

Conclusions

The BIOIMAGE trial suggests strong pharmacogenetic associations between one genetic variant of *VEGFB* (TT, rs12366035) and one genetic variant of *C5* (AA, rs12366035) genes and the VA response in week 52 to aflibercept treatment in patients with nAMD. Likewise, the results of this trial support the benefits of aflibercept treatment in nAMD previously observed in the phase III studies with a good safety profile.

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Statement of Ethics

The trial was conducted in accordance with the ethical principles of the Declaration of Helsinki and Good Clinical Practice guidelines. All ethics committees approved the trial protocol.

Conflict of Interest Statement

R.N. and A.B.J. received honoraria for consultancy and lecture fees from Bayer Healthcare Pharmaceuticals and Allergan.

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Author Contributions

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