

Safety and clinical effectiveness of intravitreal administration of bevacizumab (Lumiere®) in patients with neovascular age-related macular degeneration

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Abstract. The present study was an open-label, prospective, uncontrolled and multicenter clinical trial to investigate the safety and effectiveness of bevacizumab (Lumiere®) administered by the intravitreal route for the treatment of neovascular age-related macular degeneration (nAMD). A total of 22 patients without previous treatment with anti-vascular endothelial growth factor were recruited. Monthly therapy with 1.25 mg intravitreal bevacizumab was applied. Adverse events (AE), visual acuity (VA) and central retinal thickness (CRT) were assessed at baseline, day 1 and day 28 after each injection. A total of 87 AEs were reported; most of them were not serious (96.6%), expected (65.5%) and occurred after the third injection (56.3%). The most frequent AE was 'conjunctival hemorrhage' (29.9% of AEs), attributed to the injection procedure. Treatment was not suspended due to safety reasons in any case. After six months, a statistically significant gain of +8.2 (SD±8.8) letters and a CRT reduction of -75.50 μm (SD±120.3) were achieved with unilateral therapy. VA improvement and CRT reduction were also achieved with bilateral therapy, although to a lesser extent. The results of the present study suggested that therapy with a minimum of 3 doses of bevacizumab over a 6-month period was well tolerated and

resulted in a sustained response regarding VA improvement and CRT reduction from the beginning of therapy compared with the baseline value. The study protocol was registered at clinicaltrials.gov (ref. no. NCT03668054).

Introduction

Age-related macular degeneration (AMD) is the major cause of irreversible vision loss in individuals aged >50 years (1) and is classified as atrophic (or dry) or neovascular (wet or exudative). The neovascular variant, despite accounting for only 10% of cases, is responsible for 90% of cases of severe vision loss, defined as a visual acuity (VA) of 20/200 or worse (2,3).

It is thought that oxidative stress in the retinal pigment epithelium (RPE) leads to poor performance of its cells with the consequent formation of extracellular debris, affecting the permeability of Bruch's membrane, eventually triggering atrophy of the RPE or choroidal neovascularization. If inadequately treated, neovascular AMD (nAMD) leads to vision loss or blindness caused by leakage, hemorrhage, RPE detachments and scar formation (4,5).

Medical research has identified the vascular endothelial growth factor (VEGF) as a key pathophysiological factor in the development of nAMD, with an essential role in angiogenesis, vascular permeability and inflammatory response. Hence, intraocular inhibition of this angiogenic factor is a natural therapeutic target (6). The most used anti-VEGF drugs for nAMD are bevacizumab, ranibizumab and aflibercept.

Bevacizumab is a monoclonal antibody that inhibits all isoforms of VEGF-A and its systemic use was approved for the treatment of colorectal cancer and glioblastoma (7,8). Systemic adverse reactions associated with intravenous administration in oncology indications include thromboembolic events (e.g., myocardial infarction, cerebrovascular accident, central nervous system hemorrhage), hemoptysis, gastrointestinal

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perforation and wound healing complications (9). However, the intravitreal dose of bevacizumab is 200-400 times lower than the intravenous dose (10).

Since the introduction of intravitreal bevacizumab therapy in 2005, this medication has been frequently used by retina specialists as an off-label treatment for nAMD (11,12), although a recent survey among ophthalmologists from 20 European countries revealed a broad disparity in the off-label use of bevacizumab between countries, from non-existent (0%) to very high (97%), as well as diverging opinions expressed by governmental institutions and national ophthalmological societies (13).

Bevacizumab exhibited comparable efficacy to other anti-VEGF therapies (e.g., ranibizumab) in numerous trials conducted worldwide [CATT (14), IVAN (15), BRAMD (16), MANTA (17), GEFAL (18) and LUCAS (19) trials] with thousands of patients, and it was indicated that intravitreal bevacizumab (Avastin[®]) was not inferior to ranibizumab (Lucentis[®]) in terms of efficacy and safety (14-20). These conclusions were reaffirmed by two meta-analyses involving 5,496 and 3,665 patients with nAMD, where both anti-VEGF agents demonstrated comparable effects in terms of vision improvement and anatomical changes, as well as safety (21,22). Evidence obtained from the landmark trials along with the preferred standard practice of retina specialists supports the widespread use of bevacizumab for nAMD.

In response to the requirement for a proper adaptation, a sterile-dosage form (vial) containing 5 mg of bevacizumab in 0.2 ml injectable solution for single-dose administration has been developed. This pharmaceutical form is intended to assure intravitreal injection with minimum risk of contamination and adverse consequences, including endophthalmitis and blindness, associated with reutilization or repackaging of bevacizumab vials for oncological use. Bevacizumab also has the advantage of reducing cost of therapy when compared with other anti-VEGF alternatives, thus helping reduce the financial burden over multiple injections (23-25).

The objective of the present study was to assess the safety and clinical effectiveness of a single-dose form of bevacizumab administered via the intravitreal route in a sample of patients with nAMD naïve to anti-VEGF therapy.

Materials and methods

Study design. The present study was an open-label, interventional, single-arm, uncontrolled, prospective and multicenter clinical study to assess the safety and clinical effectiveness of intravitreal injection of bevacizumab in a sample of 22 patients with advanced nAMD (category 4 according to the Age-Related Eye Disease Study classification) (26). The study protocol was approved by the Argentinian National Administration of Drugs, Foods and Medical Technology (ANMAT; approval no. 0829/2017) and by an independent ethics committee (Comité Independiente de Ética-FEFyM, Pte. J.E. Uriburu 774 1 Piso, Ciudad Autónoma de Buenos Aires, C1027AAP, Argentina) and was performed between February 2017 and May 2018 at the three specialized research centers participating in this study (Consultorios Oftalmológicos Benisek-Ascarza, Consultorio Oftalmológico Julio Manzitti and Instituto Scorsetti). The present study was performed in

accordance with Good Clinical Practices and ethical principles laid out in the Declaration of Helsinki. The study protocol was registered at clinicaltrials.gov (ref. no. NCT03668054).

The eligibility of patients was assessed using the following inclusion criteria: i) Aged 50 years or above; ii) diagnosed with nAMD and iii) indication for antiangiogenic therapy. The exclusion criteria were as follows: i) Patients with any contraindication for bevacizumab therapy; ii) prior intravitreal and/or systemic antiangiogenic therapy; iii) nAMD in the healing period or disciform scar; iv) pregnant, breastfeeding or childbearing-aged females; v) any person with choroidal neovascularization not associated with nAMD; vi) history of retinal or intraocular surgery in the affected eye in the last 3 months; vii) vitrectomy in the affected eye; viii) any significant ocular infection having compromised or able to compromise the affected eye; ix) ocular inflammatory disease; x) myopia exceeding -8 diopters; xi) extensive subfoveal subretinal hemorrhage >2 papillary diameters; xii) coexistence of other severe ocular diseases (uncontrolled ocular hypertension, terminal glaucoma, diabetic retinopathy, retinal vein thrombosis, optic atrophy); xiii) history of stroke or myocardial infarction in the last 6 months; xiv) history of coagulopathy and xv) patients physically or mentally incompetent to perform relevant visual tests.

After obtainment of written informed consent, patients meeting the eligibility criteria underwent baseline assessments, which included VA measurement, intraocular pressure (IOP) measurement by tonometry (Goldmann Applanation Tonometer), slit-lamp biomicroscopy (Topcon SL-3E) and optical coherence tomography (SOCT Copernicus/OPTOPOL Technology). Safety follow-up was performed at day 1 post-injection [recording of adverse events (AE), vital signs, biomicroscopy and tonometry] and overall safety and therapy response were assessed at day 28 post-injection through a full ophthalmologic examination that included imaging by OCT. Data collected from patient visits were entered by the investigators in a study-specific electronic case report form.

Administration of study treatment. Bevacizumab (Lumiere[®]) is supplied as a sterile vial containing 5 mg drug in 0.2 ml of injectable solution intended for single use. After baseline assessments, patients received the first intravitreal injection of bevacizumab at a unique dose of 1.25 mg/0.05 ml and under aseptic conditions. Prior to injection, adequate anesthesia and a broad-spectrum topical microbicide were applied. Bevacizumab injections were repeated in the affected eye until 3 doses were reached. Continuation of the therapy (up to 6 injections) was decided by the investigator based on safety, tolerability and response. The time interval between injections was not less than 4 weeks. All patients, regardless of the quantity of doses received (3 doses or between 4 and 6 doses) were controlled monthly by the investigator until the finalization of the study.

Safety assessments. Drug safety was assessed in the study population that received at least one intravitreal injection of bevacizumab by monitoring the local and systemic AE pattern and severity, evolution of IOP and vital signs (systolic and diastolic blood pressure and heart rate). The primary outcome was the number of patients developing treatment-associated

AEs and safety results were compared with the published literature on the use of bevacizumab for the treatment of nAMD. All AEs were coded using the Medical Dictionary for Regulatory Activities (MedDRA) (27).

Evaluation of clinical effectiveness. Therapy response was analyzed in the study population who received at least 3 doses of bevacizumab in the affected eye by determining the VA and anatomical changes on OCT. The secondary outcomes were the number of patients developing changes in central retinal thickness (CRT) and VA, determined from the number of letters read, from baseline to months 1, 3 and 6 after therapy onset. CRT (in μm) was measured using scanned OCT images and VA was assessed using retro-illuminated, standardized Early Treatment Diabetic Retinopathy Study charts.

Statistical analysis. Descriptive statistics were used to summarize the data. Continuous variables were expressed as the median and interquartile range (IQR) or mean and standard deviation (SD) were determined, whereas categorical variables were expressed as n (%). Safety assessment included the estimation of AE rates per 100 injections of bevacizumab (at overall level and by MedDRA term/seriousness), whereas response to therapy was assessed by the change in the median value of CRT and number of letters over the study period. A non-parametric method (Friedman's test) was used to detect the overall significance, and post-hoc analyses with Bonferroni adjustment were performed to assess changes between the baseline and visits 1, 3 and 6. All tests were two-tailed and $P < 0.05$ was considered to indicate statistical significance. All analyses were performed using SAS[®] version 9.4 (SAS Institute).

Results

Patients. A total of 22 patients with a median age of 77 years (range, 61-90 years) and a female-to-male ratio of 15:7 were enrolled and received the study drug. The medical history included ophthalmic, metabolic and/or cardiovascular comorbidities frequently associated with the age group of patients presenting with nAMD (Table I).

A total of 110 bevacizumab injections were administered to 27 eyes treated (Fig. 1 and Table II). With the exception of one patient who withdrew consent after the first injection, the remaining patients (n=21 patients and 26 eyes treated) received the minimum scheme of 3 doses in the affected eye, as per the study protocol. In a subgroup of these patients (n=5 patients, 10 eyes treated), therapy was given in both eyes due to bilateral disease, with a median interval for therapy onset between eyes of 2 days, albeit with a broad dispersion (range, 1-132 days).

No protocol violations or major deviations were identified and no patients were lost to follow-up.

Safety. A total of 87 notifications of AEs were received from the patients during the 6-month follow-up period and the cumulative AE rate was 79 AE notifications for every 100 intravitreal injections (Table III). The median time to the first occurrence of an AE since therapy onset was 16 days. Most of the AEs were not serious (n=84; 96.6%), listed (i.e., AEs whose nature and severity was consistent with the product

Table I. Summary of demographics and baseline characteristics of the study population (n=22).

Characteristic	Value
Age (years)	77 (61-90)
Females	15 (68.2)
Tobacco use ^a	
Never used tobacco	8 (36.4)
Ever used tobacco	2 (9.1)
Current tobacco user	3 (13.6)
Medical history	
Cataract	20 (90.9)
Arterial hypertension	18 (81.8)
Insomnia	6 (27.3)
Hypercholesterolemia	5 (22.7)
Prostatic benign hyperplasia	5 (22.7)
Hypothyroidism	5 (22.7)
Arrhythmia	4 (18.2)
Diabetes mellitus	4 (18.2)
Right bundle branch block	3 (13.6)
Depression	3 (13.6)

^aInformation on tobacco use (smoking) was not available in 9 patients. Values are expressed as the median (range) or n (%).

information; n=57; 51.8%) and occurred after the third injection (n=49; 44.5%). The development of conjunctival hemorrhage, the most frequently reported AE in both sexes (29.9% of patients), resulted in full recovery in all cases and its occurrence was attributed to the injection procedure. In addition, most of the AEs reported whose nature and severity were not consistent with the product information were considered as not related to the study drug. Therapy with bevacizumab was not suspended due to safety reasons in any case. Non-ocular hemorrhagic events included one episode of mild hematuria (non-serious AE), which lasted for one day in a patient who had a previous history of such episodes, and was considered as not being associated with the study drug. No other non-ocular hemorrhages were reported. There were no reports of endophthalmitis (sterile or infectious), uveitis, retinal detachment, vitreous hemorrhage, anaphylactic reactions or arteriothrombotic events.

IOP variation from baseline values was assessed at all visits and no clinically relevant changes in this parameter were identified since the onset of bevacizumab therapy in the affected eye, regardless of the number of doses received (Table IV). No increase in IOP was evidenced during or immediately after the injection procedure. Likewise, non-clinically relevant IOP changes were observed in the untreated eyes. A total of 3 occurrences of IOP increment (non-serious AEs) were reported with complete recovery in all cases; two of them were considered by the investigator as not related to bevacizumab therapy and the other one as possibly related.

Only minor changes of no clinical significance in blood pressure and heart rate were observed during follow-up.

Table II. Summary of treatment schemes.

Characteristic	Unilateral treatment (17 eyes)	Bilateral treatment (10 eyes)
Total bevacizumab injections received		
n	68	42
Mean \pm SD	4.0 \pm 1.6	8.4 \pm 1.3
Received 3 loading doses (only) in affected eye	9 (52.9)	5 (50.0)
Received between 4-6 doses in affected eye	7 (41.2)	5 (50.0)

Values are expressed as n (%) unless otherwise specified. SD, standard deviation.

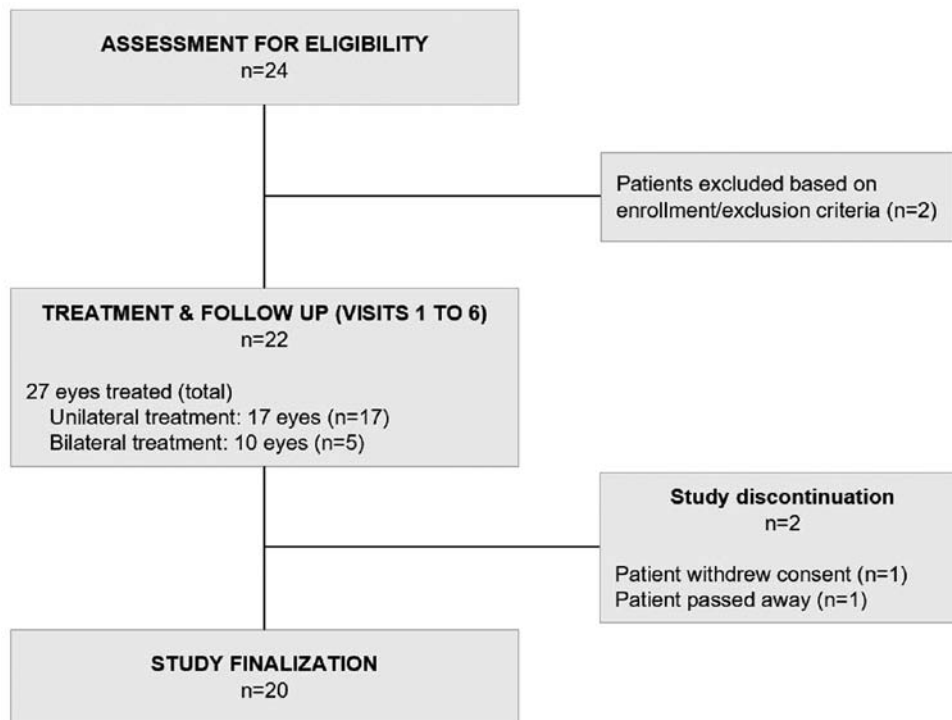


Figure 1. Flow chart of the movement of the patient in the present study. Visits 1 to 6 were performed at 1-month intervals.

Furthermore, two occurrences of arterial hypertension and one occurrence of bradycardia were notified; all of them were considered by the investigator as not serious and not related to bevacizumab therapy. In addition, no drug interactions were identified.

Clinical effectiveness. A sustained overall response was observed at 1, 3 and 6 months after therapy onset. A statistically significant improvement in VA was observed for unilateral therapy, as evidenced at the sixth month by a median (IQR) change of +8 (+6.5, +12.5) letters (mean \pm SD, +8.2 \pm 8.8 letters; range, -18 to +19 letters; $P < 0.0001$). Pairwise comparisons revealed significant differences from baseline at 1 month ($P = 0.009$), as well as at 3 and 6 months ($P < 0.0001$; Table V and Fig. 2A). Furthermore, a small gain in VA [median (IQR), +2 (-0.5, +5) letters; mean \pm SD, +1.7 \pm 5.7 letters; range, -9 to +11] was observed in the contralateral (untreated) eye at 6 months, which was not significant and of low clinical relevance (results not shown).

No significant change in VA was observed in the group receiving bilateral therapy ($P = 0.924$), with a median (IQR) gain of +3 (3, 5) letters (mean \pm SD, +5.8 \pm 11 letters; range, -6 to +24 letters) at the sixth month in the eyes treated first and no response was observed in the contralateral eye [median (IQR), -2 (-5.5, 1) letters; mean \pm SD, -2.3 \pm 6.5; range, -9 to +4; Table V and Fig. 2B and C].

Regarding the number of injections, the response to therapy observed after six months for unilateral therapy was greater in eyes that received between 4 and 6 injections [median (IQR), +10 (7, 16.5) letters; mean \pm SD, +10.8 \pm 6.8 letters; range, 0 to +19 letters] when compared to eyes that received 3 injections [median (IQR), +8 (6.7, 11) letters; mean \pm SD, +5.87 \pm 10 letters; range, -18 to +14 letters; Fig. 2D]. Likewise, a similar overall response was observed for bilateral therapy [4-6 injections group: Median (IQR), +3.5 (1.7, 4.2) letters; mean \pm SD, +2.50 \pm 3.1 letters; range, -2 to +5 letters; 3 injections group: Median (IQR), -1.5 (-6.7, 8.2) letters; mean \pm SD, +3.00 \pm 14.9 letters; range, -9 to +24 letters;

Table III. Summary of cumulative AE reporting rates.

Outcome	Frequency	AE rate (per 100 injections)
Total	87	79.1
Per seriousness category		
Not serious	84	76.4
Serious	3	2.7
Per listedness ^a		
Listed	57	51.8
Unlisted	30	27.3
Per time of occurrence		
Up to the third injection	38	34.5
After the third injection	49	44.5
Per AE term (MedDRA PT) ^b		
Ophthalmologic reactions		
Conjunctival hemorrhage ^c	26	23.6
Conjunctival hyperemia ^c	5	4.5
Eye pain ^c	4	3.6
Neovascular AMD ^{d,e}	4	3.6
Conjunctivitis/viral conjunctivitis ^e	3	2.7
Cataract/nuclear cataract ^c	3	2.7
Ocular hypertension ^c	2	1.8
Systemic reactions		
Hypertension ^c	3	2.7
Headache ^f	2	1.8

^aIndicates if the AE is (or not) described within product information. ^bOnly the most frequent AEs are described. ^cListed AE. ^dRefers to worsening of the disease. ^eUnlisted AE. ^fListed for bevacizumab by systemic use. AE, adverse event; AMD, age-related macular degeneration; MedDRA, Medical Dictionary for Regulatory Activities; PT, preferred term.

Fig. 2D]. Continuation of the therapy after the third dose was usually decided in patients exhibiting lower values of baseline VA (Table V).

A favorable response in OCT results was observed for patients receiving unilateral therapy after six months, as evidenced by a statistically significant change in the CRT ($P=0.025$). A median (IQR) reduction in the CRT of -24 (-84.2 , -3.2) μm [mean \pm SD: -75.5 ± 120.3 μm ; range, -385 to $+30$ μm] was determined for unilateral therapy (Fig. 3A and Table VI). Pairwise comparisons revealed significant differences from the baseline at 3 months ($P=0.007$) and 6 months ($P=0.027$). Comparison between the baseline and 1 month indicated no significant change in CRT ($P=0.071$).

For bilateral therapy, no significant change in CRT was obtained ($P=0.218$). In the eyes treated first, a reduction in the median (IQR) CRT of -81 (-155 , -14) μm (mean \pm SD, -86.2 ± 94.6 ; range, -204 to $+23$) was observed at six months, while no response was observed in the contralateral eye [median (IQR), $+12$ (-72 , 33.5) μm ; mean \pm SD, -29.6 ± 111.5 μm ; range, -156 to $+55$ μm ; Table VI and Fig. 3B and C].

The favorable response in OCT results was also greater in eyes receiving between 4 and 6 injections for unilateral therapy [median (IQR): -156 (-232 , -26) μm ; mean \pm SD, -156.3 ± 150.9 μm ; range, -385 to -1 μm] when compared to the eyes that received 3 injections [median (IQR), -7.5 (-39.2 , 1.7) μm ; mean \pm SD, -14.8 ± 28 μm ; range, -52 to $+30$ μm ; Fig. 3D; Table VI] and similar results were observed for bilateral therapy in eyes receiving 4-6 injections [median (IQR), -118 (-155.2 , -57.7) μm ; mean \pm SD, -95 ± 79.5 μm ; range, -204 to $+23$ μm] vs. 3 injections [median (IQR), $+4.5$ (-61.5 , 31) μm ; mean \pm SD, -35.0 ± 116.1 μm ; range, -14 to $+55$ μm]. In addition, in eyes treated unilaterally, a small reduction in the CRT [median (IQR), -1 (-8 , 3.7) μm ; mean \pm SD, -3.1 ± 13.4 μm ; range, -39 to $+12$ μm] was observed in the contralateral (untreated) eye at the sixth month, but this was not significant and of low clinical significance (results not shown).

In most patients ($n=16$ patients and 17 eyes treated), the ophthalmological assessment by slit-lamp biomicroscopy showed an improvement in the AMD throughout the treatment course with bevacizumab. Improvements encompassed both the decrease in the disease intensity and/or its change from exudative to dry AMD.

No clinically significant abnormalities were reported outside the expected alterations in patients with AMD.

Discussion

In the present study, the safety and clinical effectiveness of bevacizumab (Lumiere[®]) administered by the intravitreal route for the treatment of nAMD were assessed. The study was designed to represent the patient population usually requiring treatment for nAMD (inclusion/exclusion criteria). Safety assessment was the primary objective of the present study. In this regard, and based on data collected from the study population, no noteworthy safety risks for patients receiving a minimum of 3 doses during the 6-month period (as per the protocol) were identified. These results were consistent with those of previous studies (11,14-19,23,28-31), further confirming that bevacizumab has a favorable safety profile for its use in nAMD. None of the serious (or severe) systemic AEs that may be associated with the parenteral administration of anti-VEGF drugs were observed (14-19). Although anti-VEGF drugs are injected into the eye in small quantities, several authors have raised concerns regarding potential AE(s) resulting from the systemic suppression of VEGF (32-36). VEGF is necessary for the normal functioning of the endothelium, where it promotes vascular integrity and endothelial cell survival (37,38). Clinical experience in oncology indicated that blocking of VEGF is associated with AEs in the systemic circulation (39) and as patients with nAMD are usually of advanced age and have an increased risk of cardiovascular events, the requirement to assess cardiovascular safety is warranted. These systemic AEs include cardiovascular and arterial thromboembolic effects (e.g., stroke and myocardial infarction), but also renal and gastrointestinal effects, as well as wound healing complications. Due to this, patients with a recent history of stroke/myocardial infarction were not included in the present study and caution should be taken in patients with a history of recent cardiovascular disease or stroke, as they may have a greater risk for systemic AEs.

Table IV. Measurements of intraocular pressure (mmHg).

Item	Baseline	Difference from baseline		
		1 month (V1)	3 months (V3)	6 months (V6)
All eyes treated (overall)	12.6±2.6	-0.6±1.7	-0.2±3.1	-0.9±2.6
By treatment strategy				
Unilateral	12.6±2.9	-0.4±0.9	-0.1±3.4	-0.4±2.8
Bilateral (in eye initially treated)	12.4±1.5	-0.2±1.8	+0.8±1.3	-0.8±2.2
Bilateral (in eye contralaterally treated)	13.0±2.4	1.0±3.4	-2.0±4.2	n.d.
Received 3 loading doses (only)				
All eyes treated (overall)	13.1±2.2	-0.9±1.9	-0.9±1.9	-1.5±1.8
Unilateral therapy	13.1±2.3	-0.6±1.0	-6.1±6.1	-7.4±5.8
Bilateral therapy ^a	13.2±2.2)	-1.6±3.0	-1.3±2.6	-2.3±2.4
Received between 4 and 6 doses				
All eyes treated (overall)	10.9±4.5	-0.2±1.3	+0.6±3.0	+0.6±2.2
Unilateral therapy	11.9±3.8	-0.4±0.5	+0.4±4.0	+1.2±2.6
Bilateral therapy ^a	9.6±5.5	+0.2±1.9	+0.8±0.8	-0.3±1.3

^aIncludes eyes treated initially and contralaterally. Values are expressed as the mean ± standard deviation. V, visit; n.d., not determined.

Table V. Measurements of visual acuity (in number of letters).

Item	Eyes (n)	Baseline	Change from baseline ^c		
			1 month (V1)	3 months (V3)	6 months (V6)
All eyes treated (overall)	26	53 (30.7, 68.5)	+1 (0, 5)	+6 (0, 11)	+7.0 (3, 11)
By treatment strategy					
Unilateral	16	53.5 (26.5, 71.2)	+1.5 (0, 5.5)	+8.5 (5.5, 13.5)	+8 (6.5, 12.5)
Bilateral (in eye initially treated)	5	53 (52, 60)	-1 (-3, 0)	0 (-2, 6)	+3 (3, 5)
Bilateral (in eye contralaterally treated)	5	52 (45, 55)	+5 (-3, 5)	-1 (-2.5, 2.5)	-2 (-5.5, 1) ^a
Received 3 loading doses (only)					
All eyes treated (overall)	14	60.5 (53, 78)	+1.5 (0, 45)	+6 (4, 13)	+7.5 (0.8, 11)
Unilateral therapy	9	61 (53, 72)	+2 (1, 3)	+8 (6, 13)	+8 (6.7, 11)
Bilateral therapy ^b	5	60 (53, 84)	-1 (-3, 8)	-3 (-4.7, 3.5)	-1.5 (-6.7, 8.2)
Received between 4 and 6 doses					
All eyes treated (overall)	12	44 (28, 52.5)	+0.5 (0, 5.5)	+5 (0, 10)	+6 (3.5, 13)
Unilateral therapy	7	30 (24, 48.5)	+1 (0, 8)	+9 (2.5, 13.5)	+10 (7, 16.5)
Bilateral therapy ^b	5	52 (45, 52)	0 (-3, 5)	0 (0, 6)	+3.5 (1.7, 4.2)

Only eyes receiving 3 or more bevacizumab injections were considered for VA analysis. Values are expressed as the median (interquartile range). ^aAssessment was only performed in 3 eyes at V6. ^bIncludes eyes treated initially and contralaterally. ^cA significant improvement in VA was observed for unilateral therapy ($P<0.0001$) and pairwise comparisons showed significant differences from baseline at V1 ($P=0.009$), V3 and V6 ($P<0.0001$). VA, visual acuity; V, visit.

Another major safety concern associated with intravitreal administration is the development of severe ophthalmologic infections (e.g., infectious endophthalmitis), which were reported in previous studies (21,40). Besides a single case of bilateral non-serious viral conjunctivitis, no other ocular infections were reported in the present study. The factors towards such a favorable safety outcome would include

proper drug storage/handling at the research center, drug preparation and administration under strict aseptic conditions, use of preprocedural topical broad-spectrum antibiotics (e.g., gatifloxacin) and proper post-procedural guidance provided to the patient. Furthermore, the pharmaceutical form of bevacizumab (Lumiere[®]) as a single-dose vial avoids the possibility of drug reutilization.

Table VI. Measurements of CRT [in μm].

Item	Eyes (n)	Baseline	Change from baseline ^c		
			1 month (V1)	3 months (V3)	6 months (V6)
All eyes treated (overall)	26	266.5 (235.7, 389)	-32 (-119.2, 2.7)	-64 (-120, -9)	-25.5 (-140, -1.5)
By treatment strategy					
Unilateral	16	260 (237.2, 396.7)	-32 (-83.2, 1.2)	-57 (-93.7, -7.7)	-24 (-84.2, -3.2)
Bilateral (in eye initially treated)	5	338 (294, 417)	-111 (-122, -78)	-102 (-124, -85)	-81 (-155, -14)
Bilateral (in eye contralaterally treated)	5	251 (219, 380)	+10 (-9, 56)	-4.5 (-61.7, 3)	+12 (-72, 33.5) ^a
Received 3 loading doses (only)					
All eyes treated (overall)	14	236.5 (220, 267.7)	-6.5 (-40.5, 9.7)	-22 (-77, 9)	-7.5 (-39.2, 9.5)
Unilateral therapy	9	238 (223, 258)	-14 (-18, 2)	-9 (-50, 9)	-7.5 (-39.2, 1.7)
Bilateral therapy ^b	5	225 (219, 294)	+10 (-111, 12)	-73 (-146.2, -14.2)	+4.5 (-61.5, 31)
Received between 4 and 6 doses					
All eyes treated (overall)	12	386 (319, 499.7)	-100 (-185, -36.7)	-93.5 (-220.2, -55.5)	-125 (-201.7, -22.5)
Unilateral therapy	7	492 (313.5, 527)	-123 (-221, -58)	-120 (-256, -70.5)	-156 (-232, -26)
Bilateral therapy ^b	5	380 (338, 392)	-78 (-122, -9)	-85 (-102, -10)	-118 (-155.2, -57.7)

Only eyes receiving 3 or more bevacizumab injections were considered for CRT analysis. ^aOCT scan assessment was only performed in 3 eyes at Visit 6. ^bIncludes eyes treated initially and contralaterally. ^cA significant improvement in CRT was observed for unilateral therapy (P=0.025) and pairwise comparisons therapy showed significant differences from baseline at V3 (P=0.007) and V6 (P=0.027). Values are expressed at the median (interquartile range). OCT, optical coherence tomography; CRT, central retinal thickness; V, visit.

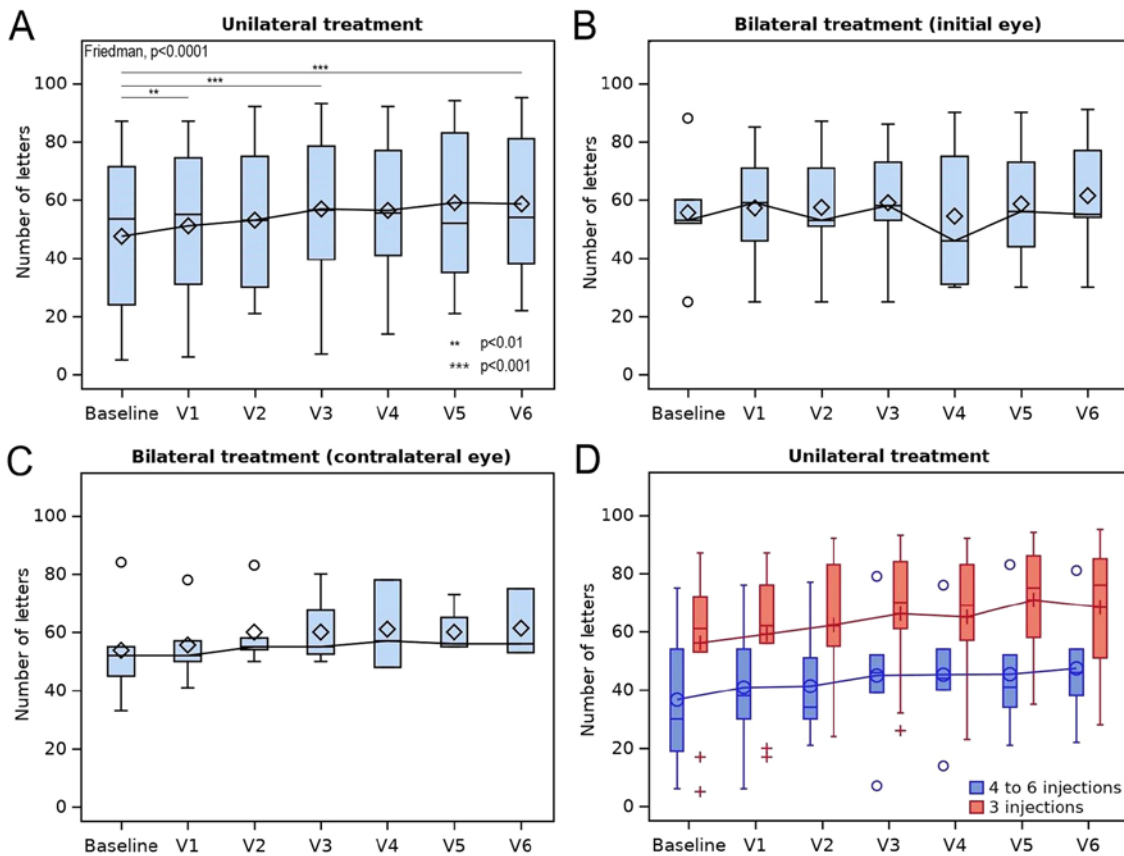


Figure 2. Evolution of the total number of letters read (y-axis) as a measure of VA from baseline through to 6 months in patients who received (A) unilateral therapy (B) bilateral therapy (eye that was initially treated), (C) bilateral therapy (eye treated contralaterally) and (D) unilateral therapy (according to the total number of injections received). Results of baseline and follow-up assessments (visits 1 to 6) are presented and all visits are 1 month apart. A significant improvement in VA was observed for unilateral therapy (P<0.0001) and pairwise comparisons showed significant differences from baseline at V1 (P=0.009), V3 and V6 (P<0.0001). The interpolation line connects the median value of number of letters between the visits and the data points (small circles/plus symbols) correspond to outliers. **P<0.01; ***P<0.001 VA, visual acuity; V, visit.

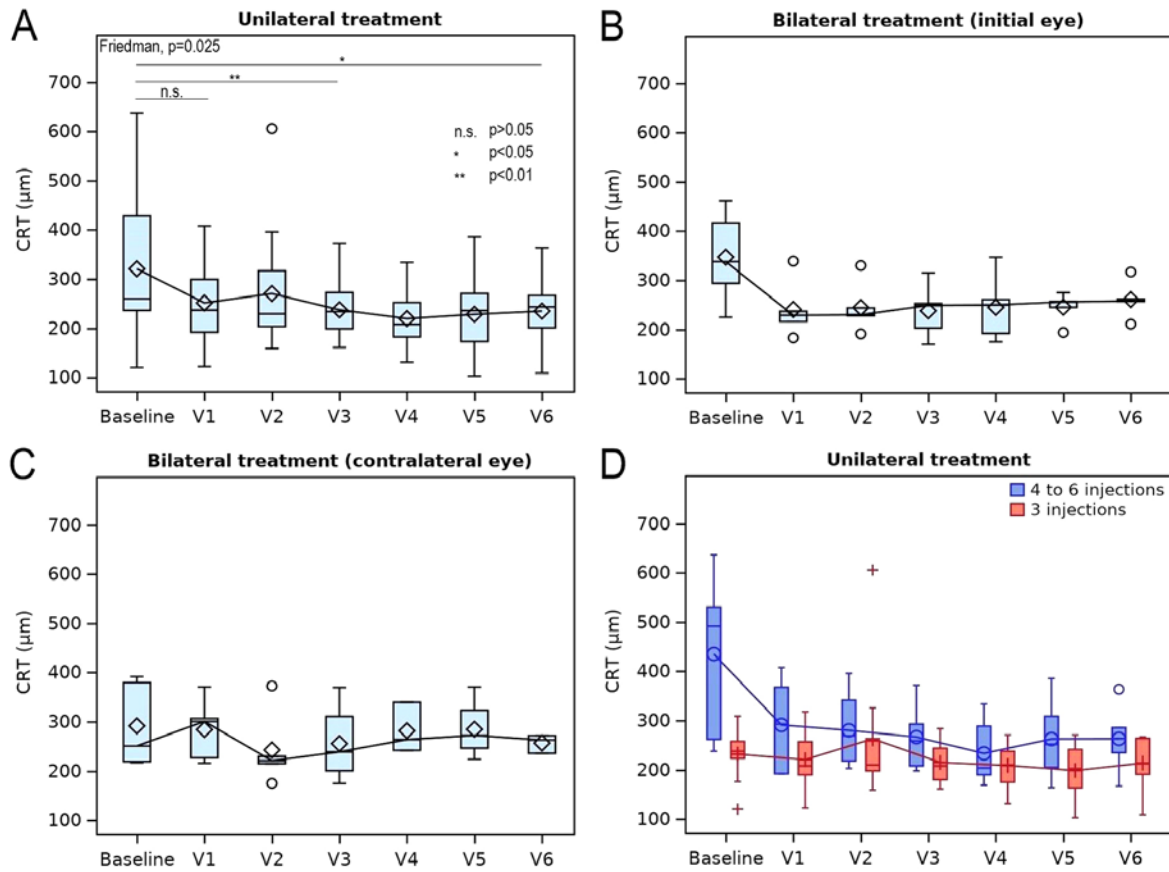


Figure 3. Evolution of CRT measured by optical coherence tomography (y-axis) from baseline through to 6 months in patients who received (A) unilateral therapy, (B) bilateral therapy (eye that was initially treated), (C) bilateral therapy (eye treated contralaterally) and (D) unilateral therapy (according to the total number of injections received). Results of baseline and follow-up assessments (visits 1 to 6) are presented and all visits are 1 month apart. Pairwise comparisons for unilateral therapy showed significant differences from baseline at V3 ($P=0.007$) and V6 ($P=0.027$). The interpolation line connects the median value of CRT between the visits and the data points (small circles/plus symbols) correspond to outliers. * $P<0.05$; ** $P<0.01$ CRT, central retinal thickness; V, visit; n.s., not significant.

Additional safety variables assessed throughout the study included IOP and vital signs (blood pressure and heart rate). Results from certain studies raised concern regarding the impact of intravitreal anti-VEGF therapy in the IOP and reported a sustained IOP elevation (i.e., IOP ≥ 21 or 22 mmHg and elevation ≥ 6 mmHg from baseline and on, at least, two consecutive visits) in 3-11% of patients who received repeated anti-VEGF injections (41-49). In the present study, patients with severe ocular hypertension were not included and the IOP was bilaterally monitored at days 1 and 28 post-injection. It was observed that the average baseline IOP values kept stable up to the sixth month and only three occurrences of non-serious AEs associated with elevated IOP were reported. Furthermore, changes observed in blood pressure and heart rate were minor and non-clinically relevant throughout the follow-up period, which is consistent with the observations reported in another study (50).

The assessment of clinical effectiveness (i.e., response to treatment) in patients receiving a minimum of 3 doses was the secondary objective of the present study and was assessed by analyzing variations over time in VA and CRT. Even though the study design and sample size did not allow for inference with estimations, treatment with intravitreal bevacizumab resulted in a favorable effect in terms of VA improvement and CRT reduction over the 6-month period. VA assessment

was achieved at the sixth month in a total of 23 eyes: 19 of these (82.6%) had either maintained (no change from baseline) or improved VA at the 6-month follow-up. These results are consistent with those of another clinical study (51).

The subgroup of patients receiving only 3 doses had less improvement in anatomical and functional outcomes when compared to those who received between 4 and 6 injections in the affected eye. The gain in VA obtained in the present study is comparable with that of previous studies and is associated with the type of treatment schedule (14,16,21,52).

Certain limitations of our study should be noted: i) Due to the small sample size, rare/uncommon AE(s) were unlikely to be detected in the present cohort (53); ii) follow-up of each patient lasted 6 months and considering that life-long treatment is required for nAMD (54), a long-term outcome assessment is not available; iii) no formal or specific assessment on the psychological impact and quality of life of anti-VEGF therapy was performed (55); iv) the present study followed a *pro re nata* treatment strategy, according to which patients were given 3 injections on a monthly basis, followed by a decision of whether to continue treatment, based on the evolution of VA and retinal thickening, safety outcomes and any other clinical finding judged by the investigator as relevant for the treatment decision. However, there are other published therapeutic strategies (e.g., treat-and-extend) that may also be

applied in clinical practice, which were not pursued in the present study (56,57).

In conclusion, the present study indicated that intravitreal injection of bevacizumab led to an improvement of VA in eyes with nAMD and with a favorable safety profile. Clinical effectiveness has been observed within 6 months of therapy onset. The sustainability of changes in retinal thickness and VA in response to bevacizumab treatment warrant further investigation and long-term follow up.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

DAB, JM and DS led the clinical trial at their study sites. AMRA, AAA, DGR, RQ, MRG, MACT and MLS evaluated the candidates according to the inclusion and exclusion criteria, treated the patients and collected the data. ES and MAT designed the protocol, implemented QA and QC systems, monitored study performance and prepared the study report. MD, MIP, FF and CL provided medical advice and pharmacovigilance services, and have also been involved in drafting and revising the manuscript for important intellectual content. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was performed in accordance with the Declaration of Helsinki and was approved by the FEFyM ethics committee from the Autonomous City of Buenos Aires (Buenos Aires, Argentina) with the reference number CI.AS-000000933/2016. Informed consent for participation in the study was obtained from all subjects.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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