Dosing Regimens of Intravitreal Aflibercept for Diabetic Macular Edema Beyond the First Year: VIOLET, a Prospective Randomized Trial

Justus G. Garweg,[1] Jana Štefanickova,[2] Carel Hoyng,[3] Tobias Niesen,[4] Thomas Schmelter,[5] Sergio Leal,[6] Sobha Sivaprasad[7] on behalf of the VIOLET Investigators

[1]Swiss Eye Institute, Rotkreuz, Berner Augenklinik am Lindenhofspital, and Department of Ophthalmology, Inselspital, University of Bern, Switzerland
[2]Department of Ophthalmology, Comenius University, University Hospital Ružinov, Bratislava, Slovakia
[3]Department of Ophthalmology, Radboud University Medical Center, Nijmegen, The Netherlands
[4]Bayer AG, Leverkusen, Germany
[5]Bayer AG, Berlin, Germany
[6]Bayer Consumer Care AG, Basel, Switzerland
[7]NIHR Biomedical Research Centre, Moorfields Eye Hospital, London, UK

Corresponding author: Professor Dr Justus G. Garweg, Berner Augenklinik am Lindenhofspital, Bremgartenstrasse 119, CH-3012 Bern, Switzerland

ONLINE SUPPLEMENT

eMethods

Study Design

Patients were randomly assigned, by central randomization via an interactive response system, to one of three intravitreal aflibercept (IVT-AFL) regimens (1:1:1): fixed treatment (injections and monitoring every 8 weeks [q8w]), treat-and-extend (T&E) treatment (injections at every visit with a treatment interval of ≥8 weeks, adjusted according to functional and anatomic criteria); or pro re nata (PRN) treatment (monitoring visits every 4 weeks; injections given as frequently as needed and decided at every visit by the investigator, based on pre-specified treatment criteria).

In the VIOLET study, all treatment groups received an initial dose of 2 mg IVT-AFL at baseline (Week 0). Due to the nature of the three different dosing regimens, the treatment groups did not have the same visit and treatment schedule, and masking of the treatments was not feasible. Visits at Week 52 (primary endpoint) and Week 100 (end of study) were mandatory for all patients.

Assessments

Visual function was assessed using the Early Treatment Diabetic Retinopathy Study (ETDRS) protocol starting at 4 m and refraction was conducted at each visit. Retinal and lesion characteristics were evaluated using spectral domain optical coherence tomography (OCT). For all visits where the OCT procedure was scheduled, images were captured and read at an independent central reading center. Intraocular pressure (IOP) was measured using applanation tonometry (Goldmann, Tonopen, or other approved alternatives). The same method of IOP measurement had to be used in each patient throughout the study. The anatomic state of the retinal vasculature of the study eye was evaluated by funduscopic examination, fundus photography, and fluorescein angiography. Fundus and angiographic images were read at screening/baseline, Week 52, and Week 100 by the investigator for the individual treatment decisions. The Diabetic Retinopathy Severity Scale (DRSS) was assessed on fundus photography by the central reading center at screening/baseline, Week 52, and Week 100.

Endpoints

Other endpoints included the mean number of IVT-AFL injections and visits, absolute best-corrected visual acuity (BCVA) from baseline to Week 100, the proportion of patients with a ≥2-step or ≥3-step improvement or worsening in DRSS (defined as patients whose DRSS category decreased or increased by 2 or 3 classes, respectively). Treatment-emergent adverse events (AEs) were defined as AEs that started after the first dose of study drug and ≤30 days after the last dose of study drug. All AEs were coded using Medical Dictionary for Regulatory Activities version 21.1. An adjudication of AEs according to the Antiplatelet Trialists' Collaboration criteria was performed.

Statistical Analysis

A sample size of 135 evaluable patients per treatment group was planned. With an expected dropout rate of approximately 17%, a total of approximately 490 patients were planned to be randomized (163 per treatment group) to ensure a sufficient power. The number of patients randomized was lower than specified in the sample-size calculation. The inclusion criteria required patients to have received 1 year of treatment with IVT-AFL before enrollment into VIOLET. It was planned that patients completing the AQUA study[1] could subsequently be enrolled into VIOLET. Patients outside the AQUA study fulfilling the eligibility criteria for this study could also be enrolled. However, despite the implementation of measures to increase enrollment, the number of eligible patients from sources other than the AQUA study remained low, and maintaining the targeted sample size of 490 randomized subjects would have resulted in a significant delay in completing the study.

The safety analysis set included all patients who received the study drug under the protocol. The full analysis set (FAS) included all randomized patients who received the study drug and had a baseline BCVA assessment and at least one post-baseline BCVA assessment; the primary statistical analysis was performed on the FAS. All variables were summarized by descriptive statistics, and categorical variables were summarized by frequency tables. The primary method for replacing missing values for all efficacy analyses was last observation carried forward. For each treatment group, the assessment of the primary endpoint was performed at Week 52. The model used was an analysis of covariance

with the baseline measure as a covariate and treatment group and stratum "10-letter gain from start of IVT-AFL to baseline (yes/no)" as a fixed factor. Because of possible unequal variances, the Kenward–Roger approximation was used to the degrees of freedom for the reference distribution. The Hochberg procedure was used to adjust for multiplicity of the two comparisons against the IVT-AFL fixed regimen. No confirmatory statistical evaluation was performed for the secondary endpoints.

1. Garweg JG, Stefanickova J, Hoyng C, et al. Vision-Related Quality of Life in Patients with Diabetic Macular Edema Treated with Intravitreal Aflibercept: The AQUA Study. *Ophthalmol Retina.* 2019;3(7):567-575.

Inclusion and exclusion criteria

Inclusion criteria

Patients who met all the following criteria were eligible for inclusion into the study:

<u>At screening and baseline</u>

1. Adults ≥18 years of age
2. The patient's history of IVT-AFL treatment met all the following:
    a. Treatment in the study eye was initiated with 5 monthly (–1 week/+2 weeks) doses of 2 mg IVT-AFL and improvements of visual and anatomic outcomes were observed and documented
    b. Following the above initiation phase, the intervals between treatments were between 6 and 12 weeks (one exception was allowed)
    c. The interval between the last two pre-study injections was ≥8 weeks, and visual and anatomic outcomes had been stable over this interval
    d. The patient received the last IVT-AFL in the study eye 8 weeks (±10 days) before the first planned treatment/randomization in this study
    e. Total prior treatment duration with IVT-AFL (i.e. from first IVT-AFL treatment ever to enrollment into this study) was 1 year or longer.

    Adherence to Criterion 2 was checked based on the available medical documentation. If no information was available, this inclusion criterion was considered as not being fulfilled and the patient could not be included in the study
3. Willingness and ability to comply with clinic visits and study-related procedures
4. Women and men of reproductive potential must have agreed to a method of highly effective contraception
5. Negative pregnancy test (serum test at screening; urine dip-stick test at baseline; women of childbearing potential only)
6. Signed written informed consent

<u>At initiation of pre-study IVT-AFL treatment</u>

The check of adherence to these criteria was based on available medical documentation. If no information was available, the respective inclusion criterion was considered as not being fulfilled and the patient could not be included in the study

7. Type 1 or 2 diabetes mellitus (DM)
8. Diagnosis of diabetic macular edema (DME) secondary to DM involving the center of the macula (defined as the area of the center subfield on OCT) in the study eye
9. Decrease in vision determined to be primarily the result of DME in the study eye
10. BCVA in the study eye of ETDRS letter score 73 to 24 (corresponding to a Snellen equivalent of approximately 20/40 to 20/320)

Exclusion criteria

<u>At initiation of pre-study IVT-AFL treatment</u>

1. Previous treatment with anti-angiogenic drugs in the study eye (e.g. pegaptanib sodium, bevacizumab, ranibizumab, or aflibercept) within the last 12 weeks before initiation of aflibercept pre-study treatment

At initiation of pre-study IVT-AFL treatment, baseline, and screening

2. History of vitreoretinal surgery and/or including scleral buckling in the study eye
3. Prior treatment of the study eye with long-acting steroids, either periocular or intraocular, in the preceding 120 days or Iluvien® intravitreal implant at any time
4. Active proliferative diabetic retinopathy, current iris neovascularization, vitreous hemorrhage, or tractional retinal detachment in the study eye
5. Aphakia in the study eye
6. Cataract surgery within 90 days before aflibercept treatment in the study eye
7. Yttrium-aluminum-garnet capsulotomy in the study eye within 30 days before IVT-AFL treatment
8. Any other intraocular surgery within 90 days of aflibercept treatment in the study eye
9. Ocular inflammation (including trace or above) or history of uveitis in the study eye
10. Vitreomacular traction or epiretinal membrane in the study eye evident biomicroscopically or on OCT that was thought to affect central vision
11. Pre-retinal fibrosis involving the macula of the study eye
12. Structural damage to the center of the macula in the study eye that was likely to preclude improvement in BCVA following the resolution of macular edema, including atrophy of the retinal pigment epithelium, subretinal fibrosis or scar, significant macular ischemia, or organized hard exudates
13. Concurrent disease in the study eye, other than DME, that could compromise visual acuity, require medical or surgical intervention during the study period, or could confound interpretation of the results (including advanced glaucoma, retinal vascular occlusion, retinal detachment, macular hole, or choroidal neovascularization of any cause)
14. Myopia of a spherical equivalent prior to any possible refractive or cataract surgery of ≥8 diopters in the study eye
15. Administration of systemic anti-angiogenic agents within 180 days before IVT-AFL treatment
16. Uncontrolled DM, as defined by glycated hemoglobin >12.0%
17. Uncontrolled blood pressure (defined as systolic blood pressure >160 mmHg or diastolic blood pressure >95 mmHg while patient was sitting, confirmed in two separate measurements)
18. Presence of any contraindications indicated in the EU commission/locally approved label for IVT-AFL

At screening and baseline:

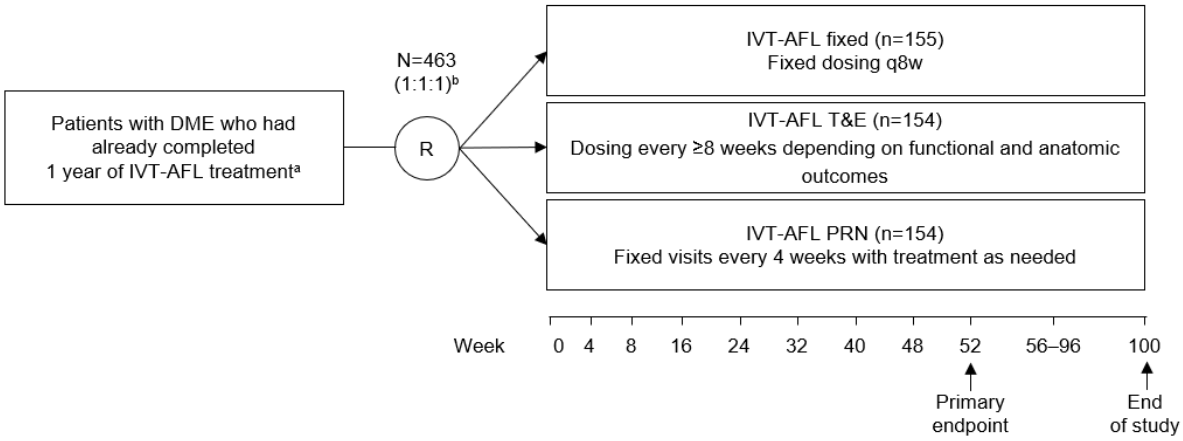
19. Evidence of infectious blepharitis, keratitis, scleritis, or conjunctivitis in either eye
20. Any ocular or periocular infection in the preceding 4 weeks in either eye
21. Filtration surgery for glaucoma in the past or likely to be needed in the future on the study eye
22. Uncontrolled glaucoma (defined as IOP >25 mmHg despite treatment with antiglaucoma medication) in the study eye
23. Allergy or hypersensitivity to fluorescein
24. Current treatment for a serious systemic infection
25. History of either cerebral vascular accident and/or myocardial infarction within 180 days before IVT-AFL treatment
26. Renal failure requiring dialysis or renal transplant
27. History of other disease, metabolic dysfunction, physical examination finding, or clinical laboratory finding giving reasonable suspicion of a disease or condition that contraindicated the use of an investigational drug, might have affected interpretation of the results of the study, or rendered the patient at high risk for treatment complications
28. Significant media opacities, including cataract, in the study eye that interfered with visual acuity, fundus photography, or OCT imaging
29. Breast-feeding women
30. Previous receipt of at least one dose of study drug under the protocol
31. Concomitant participation in another clinical study with investigational medicinal product(s). Exception: A temporal overlap with participation in Bayer study protocol 17850 was acceptable
32. Close affiliation with the investigational site, e.g. a close relative of the investigator, dependent person (e.g. employee or student of the investigational site)

List of Ethics Committees

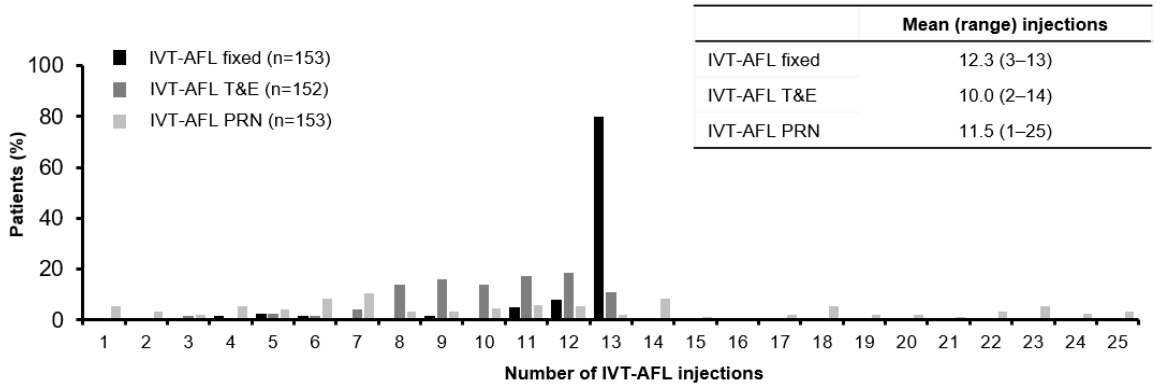
Austria	Ethikkommission der Med. Uni Wien 1090 Wien
	Ethikkommission der Medizinischen Universität Graz 8036 Graz
Canada	Advarra IRB (Formerly Cheseapeke) 21044 Columbia, MD (USA)
	Nova Scotia Health Authority Research Ethics Board Halifax, Nova Scotia, B3H 1V7
Czech Republic	Eticka komise FN KV Praha 10, 100 34
	Etická komise Fakultní nemocnice Hradec Králové 500 05 Hradec Kralové
France	Comité de Protection des Personnes (CPP) SUD-EST II 69500 Bron
Germany	Ethikkommission der Landesärztekammer Hessen Ethikkommission 60314 Frankfurt
	Ethik-Kommission der Medizinischen Fakultät der Universität zu Köln 50937 Köln
	Ethik-Kommission der Medizinischen Fakultät der Georg-August-Universität 37075 Göttingen
	Ethik-Kommission des Fachbereichs Medizin der Johann Wolfgang Goethe-Universität Frankfurt 60590 Frankfurt am Main
	Ethikkommission Technische Universität Dresden 01307 Dresden Ethik-Kommission an der Medizinischen Fakultät der Rheinischen Friedrich-Wilhelms-Universität Bonn 53105 Bonn
	Kommission für Ethik in der ärztlichen Forschung des Fachbereichs Humanmedizin der Philipps-Universität 35032 Marburg
Hungary	Medical Research Council Ethics Committee for Clinical Pharmacology (ETT-KFEB) 1051 Budapest
Italy	Comitato Etico Regione Liguria 16132 Genova
	Comitato Etico Azienda Ospedaliero Univesitaria di Cagliari 09124 Cagliari
	Comitato Etico Interaziendale AO Città della Salute e della Scienza" di Torino 10126 Torino
	Comitato Etico Indipendente c/o Fondazione PTV Policlinico 00133 Roma
	Comitato Etico Milano Area 2 20122 Milano
	Comitato Etico Area Vasta Centro 50134 Firenze
	Comitato Etico Ospedale San Raffaele 20132 Milano
	Comitato Etico Azienda Ospedaliero Univesitaria di Cagliari 09124 Cagliari
	Comitato Etico per le Sperimentazioni 35128 Padova
Lithuania	Lithuanian Bioethics Committee LT-039219 Vilnius

Poland	Komisja Bioetyczna przy Okręgowej Izbie Lekarskiej w Gdańsku 80-204 Gdańsk
Portugal	CEIC - Comissão de Ética para a Investigação Clínica 1749-004 Lisboa
Slovakia	Nezavisla Eticka Komisia Banskobystrickeho Samospravneho Kraja 974 01 Banská Bystrica
	Eticka Komisia Univerzitna Nemocnica Bratislava 851 07 Bratislava
	Eticka Komisia Fakultna Nemocnica Nitra 95001 Nitra
	Eticka Komisia Univerzitna Nemocnica Bratislava , 826 06 Bratislava
	Eticka Komisia Fakultna Nemocnica S Poliklinikou Zilina 012 07 Zilina
Spain	Comité Ético de la Investigación con medicamentos del Hospital Universitari de Bellvitge 08907 Barcelona
Switzerland	Kantonale Ethikkommission Bern (KEK) 3010 Bern
	Commission cantonale d'éthique de la recherche (CCER) 1207 Geneva
United Kingdom	London-Harrow Research Ethics Committee Bristol BS1 2NT

eFigure 1. The VIOLET Study Design



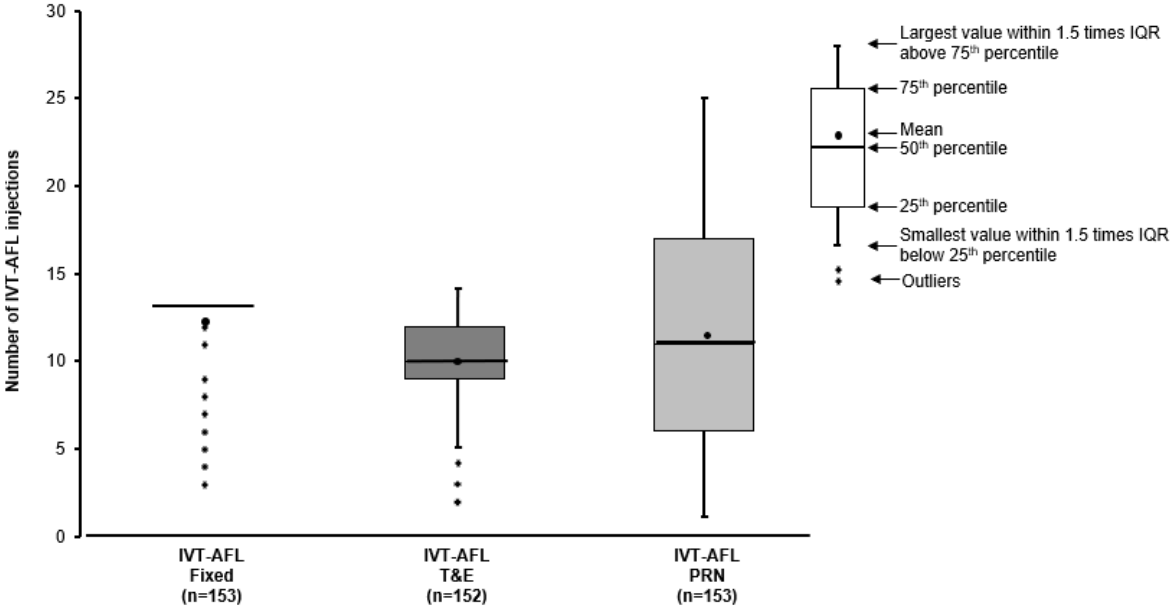
eFigure 2A. Distribution of IVT-AFL Injections Over 100 Weeks (FAS)[a]



	Mean (range) injections
IVT-AFL fixed	12.3 (3–13)
IVT-AFL T&E	10.0 (2–14)
IVT-AFL PRN	11.5 (1–25)

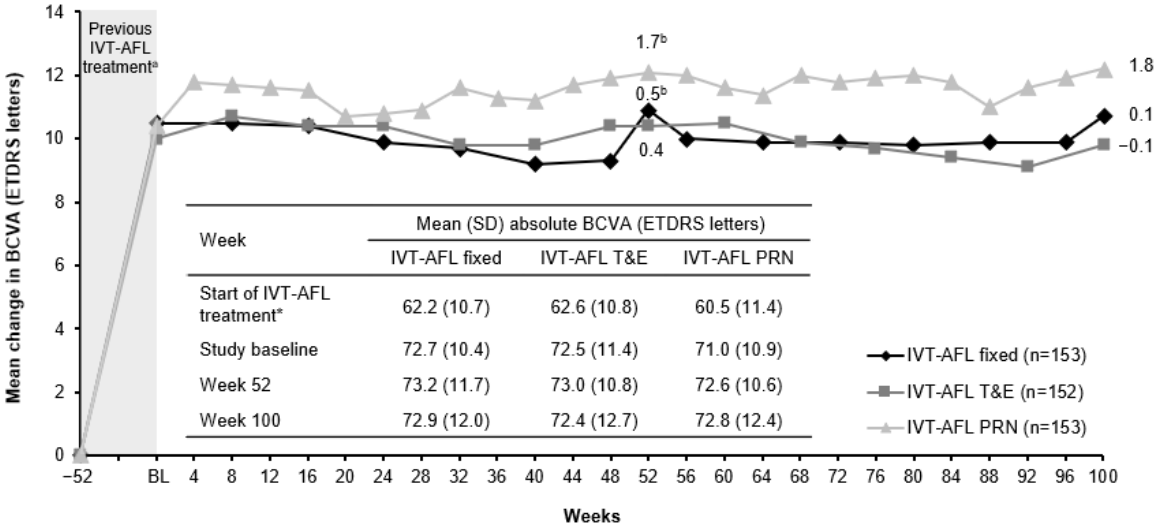
[a]Patients had previously received 1 year of IVT-AFL treatment prior to the VIOLET study baseline.
FAS, full analysis set; IVT-AFL, intravitreal aflibercept; PRN, pro re nata; T&E, treat-and-extend.

eFigure 2B. Boxplot of Treatment Exposure in Patients Treated With IVT-AFL Fixed, T&E, or PRN over 100 Weeks (FAS)[a]



[a]Patients had previously received 1 year of IVT-AFL treatment prior to the VIOLET study baseline.
FAS, full analysis set; IQR, interquartile range; IVT-AFL, intravitreal aflibercept; PRN, pro re nata; T&E, treat-and-extend.

eFigure 3. Mean Change in BCVA From the Start of the Previous IVT-AFL Treatment to Week 100 of the VIOLET Study

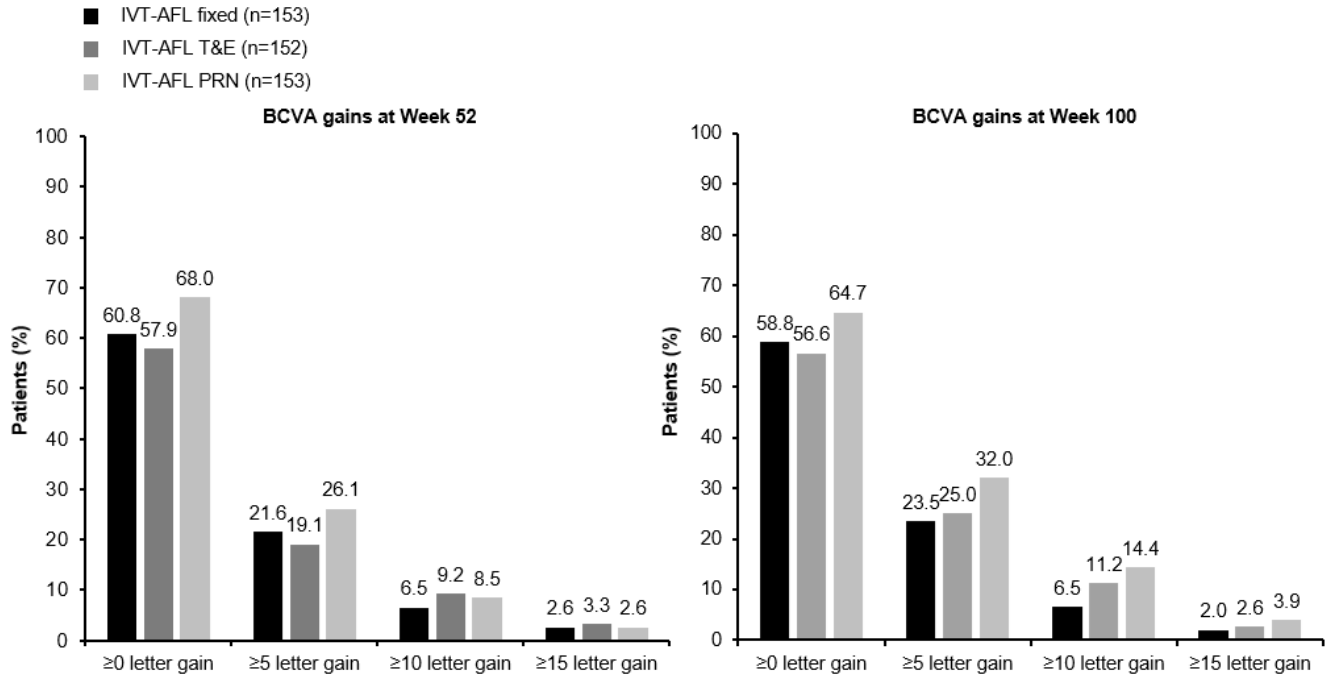


Week	Mean (SD) absolute BCVA (ETDRS letters)		
	IVT-AFL fixed	IVT-AFL T&E	IVT-AFL PRN
Start of IVT-AFL treatment*	62.2 (10.7)	62.6 (10.8)	60.5 (11.4)
Study baseline	72.7 (10.4)	72.5 (11.4)	71.0 (10.9)
Week 52	73.2 (11.7)	73.0 (10.8)	72.6 (10.6)
Week 100	72.9 (12.0)	72.4 (12.7)	72.8 (12.4)

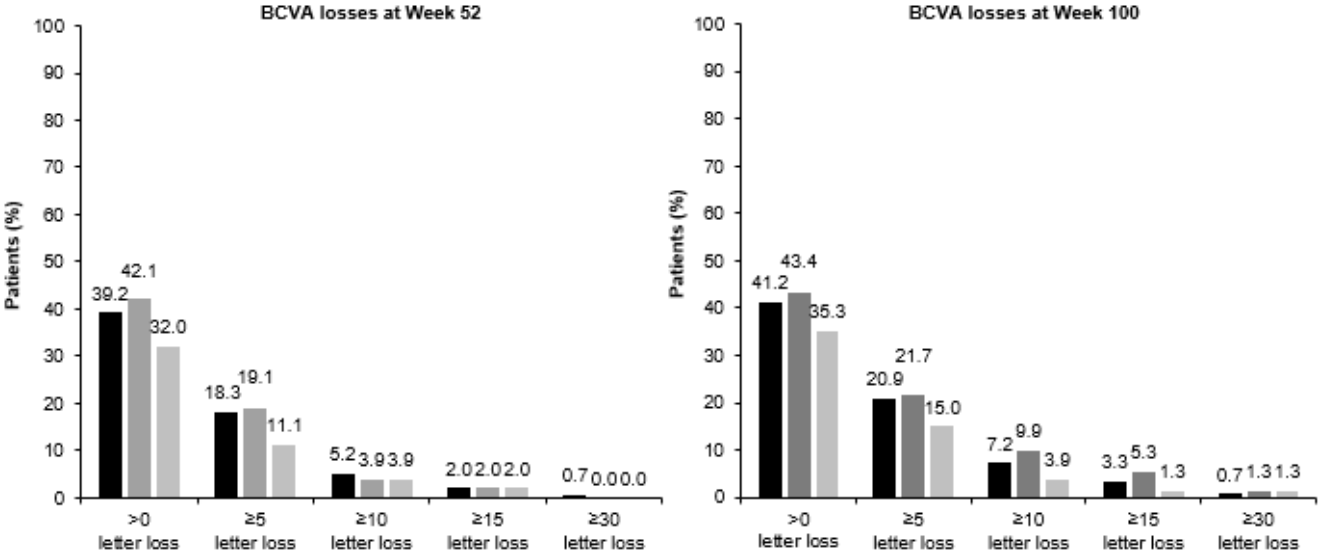
[a]Patients with DME who had already completed 1 year of IVT-AFL treatment.
[b]At Week 52 (primary endpoint), compared with IVT-AFL fixed, IVT-AFL T&E, and IVT-AFL PRN achieved a non-inferior outcome in mean BCVA change for the pre-specified margin of 4 letters (P<0.0001 for both comparisons).
Full analysis set; last observation carried forward. IVT-AFL fixed, Week 8: n=151; IVT-AFL T&E, Week 8: n=71 and Week 16: n=151; IVT-AFL PRM, Week 4, n=151.
BCVA, best-corrected visual acuity; BL, baseline; DME, diabetic macular edema; ETDRS, Early Treatment Diabetic Retinopathy Study; IVT-AFL, intravitreal aflibercept; PRN, pro re nata; SD, standard deviation; T&E, treat-and-extend.

eFigure 4. Categorical BCVA A) gains and B) losses at Weeks 52 and 100[a]

A)



B)



[a]Patients with DME who had already completed 1 year of IVT-AFL treatment.
Full analysis set. BCVA, best-corrected visual acuity; DME, diabetic macular edema; IVT-AFL, intravitreal aflibercept; PRN, pro re nata; T&E, treat-and-extend.

eTable 1. Incidence of Arterial Thromboembolic Events Defined by the APTC Criteria[a]

Number of patients (%)	IVT-AFL fixed (n=155)	IVT-AFL T&E (n=154)	IVT-AFL PRN (n=154)
Patients with ≥1 event	5 (3.2)	3 (1.9)	6 (3.9)
Non-fatal mycardial infarction	4 (2.6)	2 (1.3)	1 (0.6)
Acute myocardial infarction	1 (0.6)	2 (1.3)	1 (0.6)
Myocardial infarction	4 (2.6)	0	0
Cardiac arrest	0	1 (0.6)	0
Cardiogenic shock	1 (0.6)	0	0
Myocardial ischemia	1 (0.6)	0	0
Ventricular tachycardia	0	1 (0.6)	0
Vascular death	1 (0.6)	1 (0.6)	3 (1.9)
Cardiac arrest	0	1 (0.6)	1 (0.6)
Death	0	0	1 (0.6)
Hemorrhagic stroke	0	0	1 (0.6)
Myocardial infarction	1 (0.6)	0	0
Non-fatal stroke	1 (0.6)	1 (0.6)	2 (1.3)
Ischemic stroke	0	1 (0.6)	1 (0.6)
Lacunar stroke	1 (0.6)	0	1 (0.6)

[a]Patients with DME who had already completed 1 year of IVT-AFL treatment.
APTC, Antiplatelet Trialists' Collaboration; DME, diabetic macular edema; IVT-AFL, intravitreal aflibercept; PRN, pro re nata; T&E, treat-and-extend.

eTable 2. Causes of Death at Week 100[a]

Number of patients (%)	IVT-AFL fixed (n=155)	IVT-AFL T&E (n=154)	IVT-AFL PRN (n=154)
Non-treatment-emergent deaths	2 (1.3)	3 (1.9)	4 (2.6)
Treatment-emergent deaths[b]			
Cardiac arrest	–	1 (0.6)	1 (0.6)
Myocardial infarction	1 (0.6)	–	–
Bronchial carcinoma	–	1 (0.6)	–
Death (cause unknown)	–	–	1 (0.6)
Hepatic cancer	–	1 (0.6)	–
Hemorrhagic stroke	–	–	1 (0.6)
Suicide	–	–	1 (0.6)

[a]Patients with DME who had already completed 1 year of IVT-AFL treatment.
[b]Defined as treatment-emergent AEs that started within 30 days after the last injection and had a fatal outcome.
DME, diabetic macular edema; IVT-AFL, intravitreal aflibercept; PRN, pro re nata; T&E, treat-and-extend.