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Ranibizumab 0.5 mg treat-and-extend regimen for diabetic macular oedema: the RETAIN study

Christian Prünte,[1,2,3] Franck Fajnkuchen,[4] Sajjad Mahmood,[5] Federico Ricci,[6] Katja Hatz,[1,3] Jan Studnička,[7] Vladimir Bezlyak,[8] Soumil Parikh,[8] William John Stubbings,[8] Andreas Wenzel,[8] João Figueira,[9,10,11] and the RETAIN Study Group

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For numbered affiliations see end of article.

Correspondence to
Professor Christian Prünte, Department of Ophthalmology, Augenklinik Kantonsspital Baselland, Rheinstrasse 26, Liestal CH-4410, Switzerland; christian.pruente@ksbl.ch, christian@pruente.ch

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ABSTRACT

Aims To demonstrate non-inferiority of ranibizumab treat-and-extend (T&E) with/without laser to ranibizumab pro re nata (PRN) for best-corrected visual acuity (BCVA) in patients with diabetic macular oedema (DMO).

Methods A 24-month single-masked study with patients randomised 1:1:1 to T&E+laser (n=121), T&E (n=128) or PRN (control; n=123). All patients received monthly injections until BCVA stabilisation. The investigator decided on re-treatment in the PRN and treatment-interval adaptations in the T&E groups based on loss of BCVA stability due to DMO activity. Likewise, laser treatment was at investigator's discretion. Collectively, these features reflect a real-life scenario. Endpoints included mean average change in BCVA from baseline to months 1–12 (primary), mean BCVA change from baseline to months 12 and 24, treatment exposure and safety profile.

Results Both T&E regimens were non-inferior to PRN based on mean average BCVA change from baseline to months 1–12 (T&E+laser: +5.9 and T&E: +6.1 vs PRN: +6.2 letters; both p<0.0001). Mean BCVA change at month 24 was similar across groups (+8.3, +6.5 and +8.1 letters, respectively). The mean number of injections was 12.4 and 12.8 in the T&E+laser and T&E groups and 10.7 in the PRN group. The T&E regimens showed 46% reduction in the number of clinic visits. Over 70% of patients maintained their BCVA, with treatment intervals of ≥2 months over 24 months. Safety profile was consistent with that described in the product information.

Conclusions T&E is a feasible treatment option for patients with DMO, with a potential to reduce treatment burden. Slightly more injections were required versus PRN, likely due to the specifics of the T&E regimen applied here.

Trial registration number NCT01171976.

INTRODUCTION

Diabetic macular oedema (DMO) is the most common cause of permanent vision loss in working-age adults with diabetes.[1–3] Patients with DMO represent a heterogeneous group with varied responses to therapy that have led to individualised dosing regimens of antivascular endothelial growth factors. Currently, clinicians often practise a pro re nata (PRN) approach, wherein patients are observed monthly and treated upon signs of disease activity, or a treat-and-extend (T&E) approach, which allows incremental increase in treatment intervals with an aim to identify the longest possible treatment and visit-free interval for a given patient. The effectiveness of a PRN regimen in DMO has been established with ranibizumab 0.5 mg (Lucentis®; Genentech, South San Francisco, California, USA; and Novartis Pharma AG, Basel, Switzerland) in the long-term RESTORE and DRCR.net (protocol I) studies. In these studies, the initial best-corrected visual acuity (BCVA) improvements observed at year 1 were maintained through years 2, 3 and 5, with a reduced number of injections.[4–9] However, a PRN regimen tends to require frequent clinic visits to monitor disease status and administer treatment if needed.

The T&E approach was first introduced by Spaide and Freund in 2007 for neovascular age-related macular degeneration (nAMD), with an aim to reduce patients' treatment burden by individualising treatment intervals and reducing the number of clinic visits.[10] Studies have shown that individualised T&E regimens improve visual outcomes in nAMD and require fewer injections than those administered in a monthly regimen and fewer monitoring visits than those in a PRN regimen.[11–15]

Although the DRCR.net (protocol I) study demonstrated that DMO can be managed with less than monthly monitoring and longer treatment intervals[7–9] and the recent RELIGHT study demonstrated that bimonthly monitoring intervals were feasible in maintaining initial visual acuity (VA) gains over 12 months,[16] no T&E regimen has been evaluated in patients with DMO prior to RETAIN, the first prospective study designed to evaluate a T&E regimen in the management of DMO. The merits of two T&E regimens (with/without laser therapy) were assessed by comparing directly with the established PRN regimen. The ranibizumab PRN regimen was as per the European Summary of Product Characteristics (EU SmPC, 2011).[17] Here, we report the 24-month outcomes from the RETAIN study.

MATERIALS AND METHODS

Between September 2010 and April 2013, 372 patients with visual impairment due to DMO were enrolled at 64 centres across 13 European countries (list of investigators available in online supplementary S1) in this 24-month, phase IIIb, single-masked (VA assessor and patient were both masked to treatment assignment), controlled, three-arm parallel-group study. Written informed consent was obtained from each participating patient before study

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entry. RETAIN (registered at http://www.ClinicalTrials.gov; NCT01171976) adhered to the tenets of the Declaration of Helsinki, the International Conference on Harmonisation and Good Clinical Practice guidelines.

Patient eligibility and study treatment

The inclusion and exclusion criteria of RETAIN were comparably broader than previous confirmatory studies in DMO and aimed at inclusion of a population with relevance for real life. Patients aged >18 years with either type I or II diabetes mellitus (defined per American Diabetes Association or WHO guidelines) with glycosylated haemoglobin (HbA_{1c}) values of ≤12% at screening and an Early Treatment Diabetic Retinopathy Study (ETDRS) BCVA letter score ranging from 78 to 39, inclusive (approximate Snellen equivalent of 20/32–20/160), those with visual impairment due to focal or diffuse DMO[18] of any extent or thickness in at least one eye who were eligible for laser treatment in the opinion of the investigator, were eligible for inclusion. One eye was treated as the study eye. If both eyes were eligible, the eye with worse VA was selected as the study eye.

Patients were excluded if they showed structural damage within 0.5 disc diameter of the centre of the macula in the study eye likely to preclude improvement in VA following the resolution of macular oedema; BCVA >73 letters and central subfield thickness (CSFT) >300 µm in the study eye; any intraocular surgery in the study eye within 3 months prior to randomisation; history of vitrectomy in study eye regardless of time prior to randomisation; panretinal and focal/grid laser photocoagulation in the study eye within 6 and 3 months prior to randomisation; treatment with antiangiogenic drugs in either eye (pegaptanib sodium, anecortave acetate, bevacizumab, ranibizumab, vascular endothelial growth factor (VEGF)-Trap) within 3 months prior to randomisation; active intraocular inflammation in either eye (grade trace or above); any active infection in either eye (conjunctivitis, keratitis, scleritis, uveitis or endophthalmitis); and uncontrolled glaucoma in either eye (intraocular pressure >24 mm Hg on medications or per investigator's judgement). The complete list of exclusion criteria is presented in online supplementary S2. No additional exclusions were applied by the investigators during screening.

The patients were randomised (1:1:1) to receive either ranibizumab 0.5 mg T&E with laser (T&E+laser; n=121), ranibizumab 0.5 mg T&E without laser (T&E; n=128) or ranibizumab 0.5 mg PRN (PRN (control); n=123; online supplementary figures S1A and S1B). Details regarding patient randomisation are available in online supplementary file S3. All three treatment groups received monthly ranibizumab 0.5 mg until BCVA was stabilised (no change in BCVA over three consecutive months with treatment). In the ranibizumab T&E+laser group, patients received laser treatment on day 1, after which laser could be readministered based on the ETDRS guidelines. Laser treatment was at the discretion of the investigator, reflecting a real-life scenario, with a 3-month minimum interval recommended between treatments (see online supplementary figure S1B and file S4).

T&E design

The T&E regimen allowed the incremental extension of intertreatment intervals based on disease stability; VA loss due to disease recurrence triggered a return to monthly injections until VA stability was re-established. This conservative approach was chosen owing to lack of experience with T&E in DMO when the RETAIN study was designed. For the same reason, the maximal length of an intertreatment interval was capped at 3 months.

In detail, patients in all treatment groups received monthly ranibizumab 0.5 mg injections for at least three consecutive months until BCVA stability was achieved. BCVA stability judgement was at the discretion of the assessing clinician with no prespecified criteria. At the visit when BCVA stability was recorded, no treatment was administered. Patients randomised to either of the T&E groups were then scheduled for treatment at the next visit, that is, the treatment interval was extended to 2 months. If the patient's vision remained stable after these two months, the treatment interval was extended to 3 months, and this interval length was maintained till the patient's vision remained stable (figure 1). During treatment intervals of >1 month, patients continued study visits during the intervening months solely to maintain masking, that is, no treatment was given and no adaptation of the intertreatment interval was allowed. For PRN group patients, monthly monitoring visits were scheduled after initial confirmation of BCVA stability, and treatment reinitiated by loss of VA due to disease activity. Further details on study visits, laser treatment and re-treatment criteria are provided in online supplementary file S4.

Objectives

The primary objective was to demonstrate non-inferiority (four-letter margin) of the T&E regimen with/without laser to the PRN regimen with respect to mean average change in BCVA from baseline to month 1 through month 12. If non-inferiority was established, superiority of the T&E regimens was evaluated. Secondary objectives included the evaluation of the mean average change in BCVA from baseline to month 1 through month 24; mean change in BCVA and change in CSFT (average retinal thickness of the circular area with 1 mm diameter around the foveal centre) from baseline to months 12 and 24; mean number and pattern of treatments over 12 and 24 months; impact of laser on the number of re-treatments in the T&E groups and incidence of ocular and non-ocular adverse events (AEs) and serious AEs (SAEs).

Study assessments and analysis

Best-corrected visual acuity

A certified evaluating investigator (masked to the treatment assignment) used ETDRS-like VA testing charts at a starting distance of 4 m to assess BCVA of the study eye. Additionally, this investigator performed other study efficacy assessments and judged the presence or absence of BCVA stability and disease activity or recurrence.

Optical coherence tomography

Spectral or time-domain optical coherence tomography (OCT) was performed at every study visit. The same device was to be used for a given patient throughout the study. All images were assessed by trained and qualified experts at the sites; no central reading centre was involved. Change in CSFT was analysed as change from baseline in per cent.

Treatment exposure

The number of ranibizumab injections and laser treatments were recorded for each treatment group. Other endpoints included average treatment interval, that is, the interval (in months) between visits at which treatment was administered to the study eye, from the first treatment visit after initial BCVA stability was confirmed up to month 24, and the total number of visits

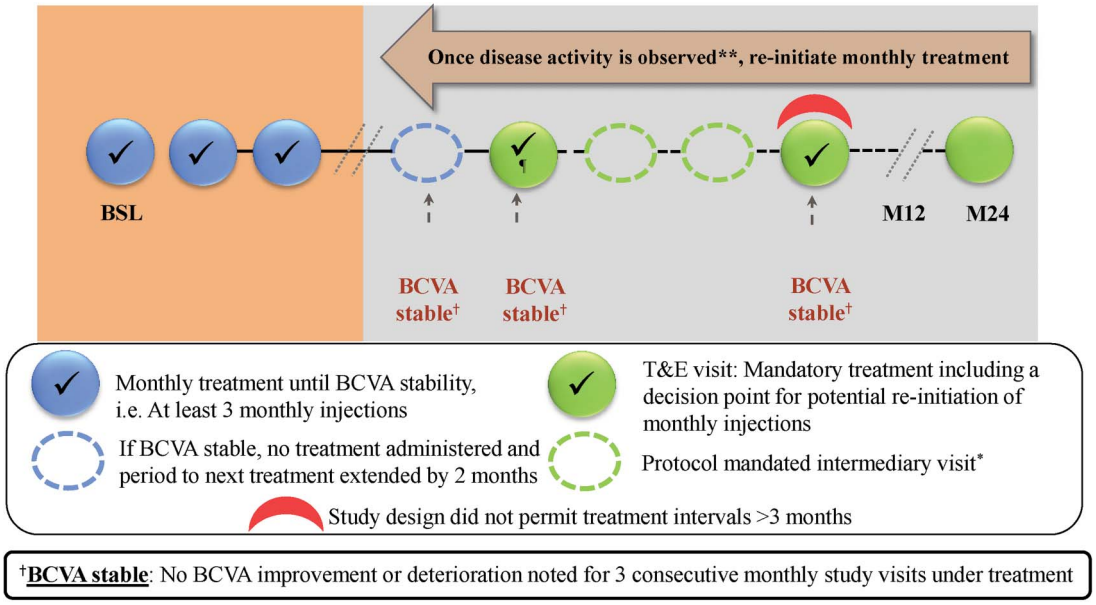


Figure 1 The treat-and-extend (T&E) treatment algorithm. *Scheduled between the T&E visits where no study treatment was administered and no decision for study treatment was made. For the pro re nata (PRN; control) regimen, each monitoring visit was also a potential treatment visit. †Best-corrected visual acuity (BCVA) stable: no BCVA improvement or deterioration noted for three consecutive monthly study visits under treatment. **Patient's BCVA worsened due to diabetic macular oedema (DMO) disease activity. ¶First visit followed 1 month after the visit at which stabilisation (at month 3) was confirmed. BSL, baseline; M, months.

scheduled for treatment after the visit with initial BCVA stability up to month 24.

Safety

Safety assessments included the incidence of ocular and non-ocular AEs and SAEs, their frequency and relationship to treatment/ocular injection. All AEs were summarised by system organ class, based on the preferred term, and were grouped per the standardised *Medical Dictionary for Regulatory Activities*.

Statistical analysis

A sample size of 104 patients per treatment group had >90% power to establish non-inferiority at a four-letter margin for at least one of the two T&E regimens compared with the PRN regimen in terms of the mean average change in BCVA based on a one-sided significance level of 0.0125, assuming a treatment difference of 1 letter, SD of 10 letters and underlying normal distribution for an unstratified Mann–Whitney test. The primary analysis was conducted after patients had completed the month 12 visit using the full analysis set (FAS), which comprised all randomised patients who received at least one application of the study treatment (ranibizumab or laser) and had at least one post-baseline BCVA assessment. The mean value/last observation carried forward approach was used to impute missing postbaseline data. Patients were analysed according to the treatment assigned at randomisation (intent-to-treat principle). Hypotheses of the primary objective for non-inferiority and superiority were tested using a sequentially rejective multiple testing procedure that protects the multiple one-sided alpha level of 0.025.[19] The safety analysis was conducted on the safety set that comprised all patients who received at least one application of study treatment and had at least one postbaseline safety assessment. Patients were assigned to treatment groups according to the actual treatment they received. Further details are provided in online supplementary file S5.

RESULTS

Patient demographics and baseline characteristics

Of the 372 enrolled patients, 332 (89.2%; mean age, 63.7 years; males, 62.4%) completed the study. The efficacy analysis was performed on the FAS (n=359), and the safety analysis on the safety set (n=370; online supplementary figure S2).

AEs and withdrawal of consent were the most common reasons for discontinuation across all treatment groups (see online supplementary figure S2). Overall, patient demographics as well as baseline disease and ocular characteristics were generally well balanced across treatment groups (table 1). Patients had mild to moderate vision loss at baseline (mean BCVA: 63.4 letters; WHO, International Classification of Functioning, Disability and Health; online supplementary file S6). Further details are provided in table 1.

Efficacy

Best-corrected visual acuity

The primary endpoint was met, with both the T&E regimens (T&E ranibizumab+laser and T&E ranibizumab) being non-inferior to PRN with respect to mean average change (±SD) in BCVA from baseline to month 1 through month 12 (5.91 (±5.53) letters and 6.14 (±5.71) letters vs 6.20 (±6.01) letters, respectively; online supplementary figure S3 and table S2). The upper limits of the 97.5% CIs for differences in least-squares

Table 1 Key patient demographics and baseline diabetes and ocular characteristics (randomised set)

	T&E ranibizumab 0.5 mg+laser n=121	T&E ranibizumab 0.5 mg n=128	PRN ranibizumab 0.5 mg n=123
Characteristic			
Mean age±SD, years	63.7±9.1	63.0±9.8	64.5±9.7
Gender, n (%)			
Male	78 (64.5)	77 (60.2)	77 (62.6)
Female	43 (35.5)	51 (39.8)	46 (37.4)
Race, n (%)			
Caucasian	114 (94.2)	126 (98.4)	117 (95.1)
Black	3 (2.5)	1 (0.8)	3 (2.4)
Asian	1 (0.8)	0 (0.0)	1 (0.8)
Other	3 (2.5)	1 (0.8)	2 (1.6)
Mean HbA_{1c}±SD, %	7.8±1.4	7.9±1.3	8.0±1.2
Diabetes, n (%)			
Type I	10 (8.3)	12 (9.4)	10 (8.1)
Type II	111 (91.7)	116 (90.6)	113 (91.9)
DMO, n (%)			
Focal	36 (29.8)	28 (22.0)	32 (26.0)
Diffuse	62 (51.2)	72 (56.7)	70 (56.9)
Mean time since DMO diagnosis (±SD), years	2.54±3.2	2.64±3.1	2.53±3.0
Time since first diagnosis of DMO (categorised), months, n (%)			
≤3	16 (13.2)	15 (11.8)	18 (14.6)
>3–<12	32 (26.4)	32 (25.2)	28 (22.8)
≥12	70 (57.9)	79 (62.2)	77 (62.6)
Mean CSFT±SD, µm	480.7±165.0	452.4±131.2	432.5±129.9
Mean BCVA±SD (letters)	61.7±12.2	63.9±10.8	64.7±10.2
Range (BCVA ETDRS letters)	23–80	34–83	39–84

Randomised set included all randomised patients (ie, those assigned a randomisation number).
BCVA, best-corrected visual acuity; CSFT, central subfield thickness; DMO, diabetic macular oedema; ETDRS, Early Treatment Diabetic Retinopathy Study; HbA_{1c}, glycosylated haemoglobin; PRN, pro re nata; T&E, treat-and-extend.

mean for the PRN versus T&E groups were <4 letters for both comparisons. The superiority of T&E groups over the PRN group was not established (see online supplementary figure S3 and table S2).

There was no statistical difference between the two T&E groups in terms of the average change in BCVA from baseline to month 1 through month 12 and 24 treatment periods (see online supplementary figure S3 and table S2). In all treatment groups, the mean BCVA increased from baseline during the first four months of treatment by ~five letters, with subsequent steady increase of 1–3 letters over the following 20 months. At month 24, mean BCVA change from baseline improved across all treatment groups (figure 2A and table 2).

Central subfield thickness

CSFT was reduced in all treatment groups at month 12, which persisted until month 24 (figure 2B and table 2). The mean CSFT per cent change from baseline by OCT machine type is provided in online supplementary figure S4.

Treatment exposure

Ranibizumab 0.5 mg injections and laser treatment

Over the initial 12 months, all treatment groups received a median of seven injections; over 24 months, median number of injections was 12 in both T&E groups and 10 in the PRN group. A majority of T&E+laser group patients (77.8%) received only one laser treatment over 24 months (table 3).

Average interval between treatments

After the visit with initial BCVA stability up to month 24, >70% patients in the T&E groups maintained their initial BCVA stability with intertreatment intervals of ≥2 months (table 3).

Number of visits scheduled for treatment

After the visit with initial BCVA stability up to month 24, the mean number of scheduled treatment visits was 9.0 and 8.9 for the T&E groups (with/without laser, respectively) and 16.6 for the PRN group (table 3).

Safety profile

Adverse events

Non-ocular and ocular AEs were reported in approximately 70% and 39% patients, respectively, across all treatment groups. The most frequent non-ocular and ocular AEs are listed in online supplementary table S1, with a majority being of mild to moderate intensity. Discontinuations from the study due to ocular and non-ocular AEs are shown in online supplementary table S2.

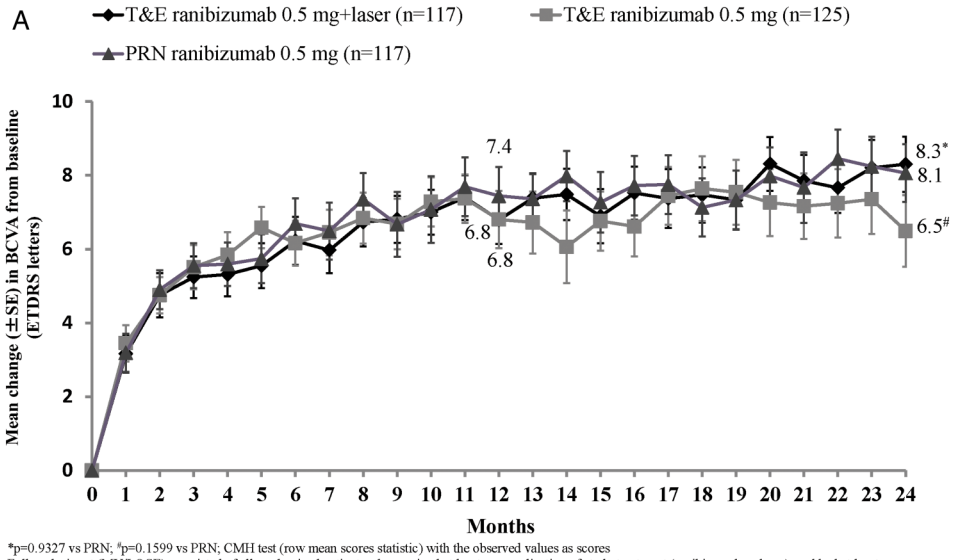
Serious adverse events

The overall incidence of ocular and non-ocular SAEs was low and similar across all treatment groups. Ocular SAEs in the study eye were reported in three patients in the T&E groups (vitreous haemorrhage and endophthalmitis in two T&E+laser group patients and periorbital haematoma in one T&E group patient), and no ocular SAEs were reported in the PRN group (see online supplementary table S3). Overall, at least one non-ocular SAE was reported in 85 patients during the study (33, 29 and 23 in the T&E+laser, T&E and PRN groups, respectively). The most commonly reported non-ocular SAEs are listed in online supplementary table S3. Most of the non-ocular SAEs were not suspected to be related to study drug and/or ocular injection.

Overall, seven deaths were reported: two (1.6%) in the T&E +laser group, four (3.2%) in the T&E group and one (0.8%) in the PRN group. Only two deaths were suspected to be treatment-related by the investigator (myocardial infarction in the T&E+laser group and cerebrovascular accident in the PRN group); details are provided in online supplementary table S3.

DISCUSSION

Findings from the RETAIN study show that both T&E regimens, with/without laser, were non-inferior to a PRN regimen and resulted in improvement and maintenance of VA in patients with DMO over 24 months. Overall, efficacy findings in patients with mild to moderate vision loss at baseline (mean VA: 63.4 ±11.15 letters) in RETAIN were consistent with findings from the RESTORE study, which included a similar patient population in terms of baseline VA (63–65 letters in both ranibizumab groups with/without laser).[4] A retrospective analysis from 1616 patients with DMO across nine phase II or III randomised clinical trials (eg, RETAIN, RESTORE, RIDE, RISE, VIVID, VISTA) of ranibizumab 0.5 mg and aflibercept 2 mg has demonstrated that, regardless of the dosing regimen and anti-VEGF compound, the mean VA at 12 months plateaued at about 70 (68.5–73.0) letters. This analysis revealed that greater BCVA gains are observed in patients with poor vision at baseline and vice versa. While in this comparative analysis the RESTORE and



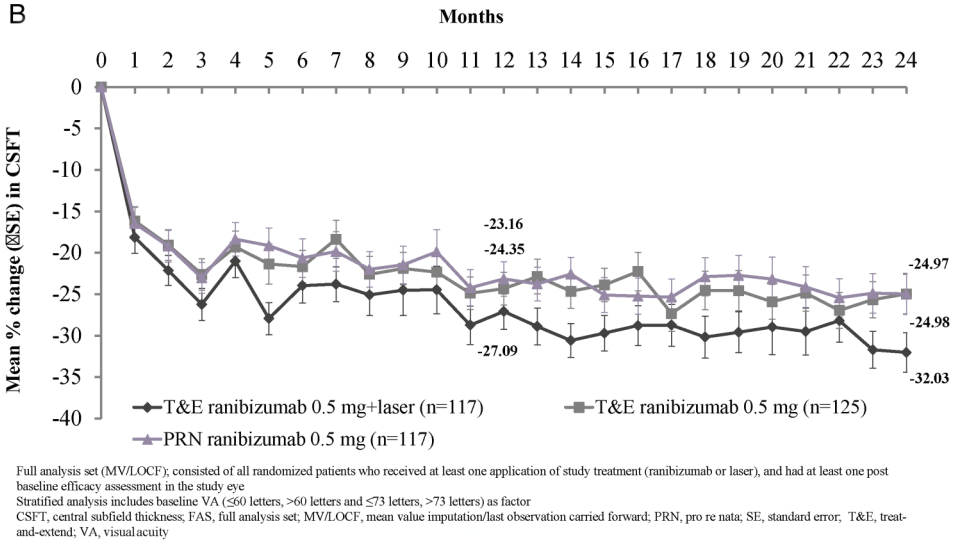


Figure 2 (A) Mean change in best-corrected visual acuity (BCVA) from baseline to months 12 and 24 (full analysis set (FAS)-mean value imputation/last observation carried forward (MV/LOCF)). *p=0.9327 versus pro re nata (PRN); #p=0.1599 versus PRN; Cochran–Mantel–Haenszel (CMH) test (row mean scores statistic) with the observed values as scores. (B) Mean percentage change in central subfield thickness (CSFT) from baseline over time (FAS-MV/LOCF). In (A) and (B), FAS (MV/LOCF) comprised all randomised patients who received at least one application of study treatment (ranibizumab or laser) and had at least one postbaseline efficacy assessment in the study eye. Stratified analysis included baseline visual acuity (≤60 letters, >60 and ≤73 letters and >73 letters) as factors. ETDRS, Early Treatment Diabetic Retinopathy Study.

RETAIN trials demonstrated the lowest gain (approximately seven letters) in BCVA for each of the PRN and T&E arms, the mean baseline BCVA in RESTORE and RETAIN trials featured among the highest. In contrast, patients in RIDE and RISE had the lowest baseline BCVA but achieved the highest BCVA gains of about 12.0 letters with monthly ranibizumab 0.5 mg. Though these BCVA gains in RIDE and RISE were reported at 24 months, they were comparable to gains observed at 12 months (Dugel PU, Hillenkamp J, Sivaprasad S, *et al*. Unpublished work. Baseline visual acuity strongly predicts visual acuity gain in patients with DME following anti-VEGF treatment across trials in DME. Retina). Thus, considering baseline BCVA effects and the apparent ceiling effect observed for anti-VEGF therapy of DMO, the BCVA results across all three regimens applied in RETAIN are comparable to results obtained with monthly regimen or other anti-VEGF agents.

The T&E in RETAIN was associated with a slightly higher number of injections (mean 12.4 and 12.8 over 24 months for the T&E+laser and T&E groups, respectively) compared with the PRN group (10.7). This increase was most likely due to the constraints of the protocol: (1) over 24 months, the T&E regimen required a minimum of 10 injections versus a minimum of three injections with PRN; (2) the maximal treatment-free interval was capped at 3 months in the T&E regimen, but the treatment intervals could have been extended to beyond 3 months for certain patients, as indicated by 18% patients who had at least one interval of >3 months in the PRN group; and (3) any unsuccessful attempt to extend the treatment-free interval required the patient to return to monthly injections, which is more conservative than the T&E regimen described previously for nAMD.[11–15 20] This conservative approach was selected when the RETAIN protocol was designed so as not to put patients with DMO at risk of vision loss. Today, a stepwise reduction of the treatment interval for patients with disease recurrence would be preferred over returning to monthly treatment, and the maximal interval would likely be extended to >3 months. Nevertheless, the T&E regimens in this study led to a substantial reduction of ~46% in the number of clinic visits up to month 24 versus the PRN regimen.

Importantly, in a clinical setting, the treatment visit with T&E regimen is predefined; thus, the patient is aware of receiving the treatment at the next clinic visit, can prepare mentally for

Table 2 BCVA and CSFT outcomes at months 12 and 24 (FAS-MV/LOCF)

	T&E ranibizumab 0.5 mg+laser n=117	T&E ranibizumab 0.5 mg n=125	PRN ranibizumab 0.5 mg n=117
Month 12 outcome			
Mean average change in BCVA letter score from baseline to months 1–12 (primary endpoint)*			
Mean±SD	5.91±5.532	6.14±5.717	6.20±6.005
Median (range)	5.00 (−7.0 to 29.0)	5.50 (−13.6 to 24.0)	5.83 (−22.8 to 20.2)
95% CI for mean†	4.90 to 6.92	5.13 to 7.16	5.10 to 7.30
Assessment of non-inferiority to PRN			
Difference in LS means‡	0.39	0.19	–
95% CI for difference	−1.03 to 1.81	−1.21 to 1.59	–
One-sided p value (CMH transformed)§	<0.0001	<0.0001	–
Change in BCVA letter score from baseline to month 12			
Mean±SD	6.79±6.999	6.80±8.726	7.44±8.457
Median (range)	6.00 (−9.0 to 35.0)	6.00 (−35.5 to 26.0)	7.00 (−46.0 to 28.0)
95% CI for mean†	5.50 to 8.07	5.25 to 8.34	5.89 to 8.98
Per cent change in CSFT from baseline to month 12, μm			
Mean±SD	−27.09±22.992	−24.35±22.027	−23.16±22.362
Median (range)	−26.95 (−82.7 to 22.8)	−24.00 (−68.6 to 38.0)	−22.90 (−73.7 to 53.1)
95% CI for mean	−31.32 to −22.87	−28.27 to −20.43	−27.27 to −19.05
Comparison vs PRN			
Difference in LS means (vs PRN)	0.82	−0.02	–
95% CI for difference	−4.56 to 6.20	−5.25 to 5.22	–
p Value¶	0.2178	0.7384	–
Month 24 outcome			
Mean average change in BCVA letter score from baseline to months 1–24			
Mean±SD	6.78±5.986	6.58±7.070	6.97±6.430
Median (range)	6.04 (−8.6 to 31.7)	6.33 (−28.0 to 21.7)	6.71 (−20.8 to 25.0)
95% CI for mean	5.68 to 7.87	5.33 to 7.83	5.79 to 8.15
Comparison vs PRN			
Difference in LS means	0.30	0.54	–
95% CI for difference	−1.32 to 1.92	−1.06 to 2.13	–
Two-sided p value¶	0.6920	0.5186	–
Change in BCVA letter score from baseline to month 24			
Mean±SD	8.30±8.129	6.49±10.854	8.06±8.462
Median (range)	8.00 (−19.0 to 41.0)	7.00 (−50.0 to 26.0)	8.00 (−27.0 to 32.0)
95% CI for mean	6.81 to 9.79	4.57 to 8.41	6.51 to 9.61
Per cent change in CSFT from baseline to month 24, μm			
Mean±SD	−32.03±25.628	−24.98±26.414	−24.97±26.678
Median (range)	−34.35 (−82.7 to 87.8)	−27.40 (−77.2 to 69.5)	−26.55 (−76.4 to 74.0)
95% CI for mean†	−36.75 to −27.32	−29.68 to −20.29	−29.88 to −20.07
Comparison vs PRN			
Difference in LS means (vs PRN)	3.01	−1.23	–
95% CI for difference	−3.26 to 9.29	−7.34 to 4.87	–
Two-sided p value¶	0.0467	0.9360	–

Months 12 and 24 outcomes: FAS consisted of all randomised patients who received at least one application of study treatment (ranibizumab or laser), and had at least one postbaseline efficacy assessment in the study eye.
*Assessments of superiority of T&E groups vs PRN group using nominal one-sided p values for untransformed CMH tests showed that the comparisons of T&E ranibizumab+laser vs PRN (p=0.7064) and T&E ranibizumab vs PRN (p=0.6052), respectively, were statistically non-significant (>0.0125 for both comparisons).
†Two-sided 95% CI are based on t-distribution.
‡Average change from baseline to months 1–12 in BCVA analysed using ANOVA with stratified baseline BCVA and treatment as factors.
§CMH test uses row mean scores statistic.
¶p Values are from two-sided stratified CMH test using the row mean scores statistic.
ANOVA, analysis of variance; BCVA, best-corrected visual acuity; CMH, Cochran–Mantel–Haenszel; CSFT, central subfield thickness; FAS, full analysis set; LS, least square; MV/LOCF, mean value imputation/last observation carried forward; PRN, pro re nata; T&E, treat-and-extend.

treatment and make adjustments in their schedule if necessary. Moreover, the clinic can pre-prepare the theatre schedule, injections and staffing, thus optimising time and resources.

In the RETAIN study, the frequency distribution of the average interval between treatment visits showed that a T&E regimen adapts to a patient's needs. During the 24-month study period, the proportion of patients who had an average interval between treatments of 2 and 3 months from initial BCVA stabilisation ranged from 36% to 44% in the T&E+laser group and 39–44% in the T&E group (table 3). Thus, in the absence of DMO activity, ~44% patients in the T&E groups were able to maintain vision and extend the treatment-free interval up to 3 months. In fact,

Table 3 Number of ranibizumab injections and laser treatments, number of visits scheduled for treatment from months 3 to 24 and treatment intervals up to month 24 (safety set)

	T&E ranibizumab 0.5 mg+laser n=126	T&E ranibizumab 0.5 mg n=126	PRN ranibizumab 0.5 mg n=118
Number of injections (up to month 24)			
Total	1563	1607	1259
Mean±SD	12.4±3.8	12.8±3.7	10.7±5.6
Median (range)	12 (3–23)	12 (3–23)	10 (1–24)
Frequency of injections, n (%)			
1–3	1 (0.8)	1 (0.8)	10 (8.47)
4–6	6 (4.76)	3 (2.38)	20 (16.95)
7–9	7 (5.56)	7 (5.56)	27 (22.88)
10–12	66 (52.38)	65 (51.59)	22 (18.64)
13–15	23 (18.25)	23 (18.25)	14 (11.86)
16–18	12 (9.52)	17 (13.49)	12 (10.16)
19–21	9 (7.14)	8 (6.35)	8 (6.78)
22–24	2 (1.59)	2 (1.59)	5 (4.23)
Number of laser treatments (up to month 24)			
Total	146	–	–
Mean±SD	1.2±0.66	–	–
Median (range)	1 (0–4)	–	–
Frequency of laser, n (%)			
0	8 (6.3)*	–	–
1	98 (77.8)	–	–
2	15 (11.9)	–	–
3	2 (1.6)	–	–
4	3 (2.4)	–	–
Number of visits scheduled for treatment† after the visit with initial BCVA stability up to month 24			
Total‡	1131	1105	1828
Mean±SD	9.0±4.30	8.9±3.81	16.6±4.28
Median (range)	8 (1–21)	8 (2–20)	18 (1–20)
Average interval between treatment (months)§			
N	123	123	90
Mean±SD	2.468±0.954	2.299±0.616	2.828±2.644
Median (range)	2.5 (0.93–7.95)	2.4 (0.98–3.79)	2.145 (0.68–19.63)
Frequency, n (%)¶			
1 month (16–45 days)	18 (14.6)	20 (16.3)	22 (24.4)
2 months (46–75 days)	44 (35.8)	48 (39.0)	36 (40.0)
3 months (76–105 days)	54 (43.9)	54 (43.9)	16 (17.8)
>3 months (≥106 days)	7 (5.7)	1 (0.8)	16 (17.8)

Safety set comprised all patients who received at least one active application of study treatment and had at least one postbaseline safety assessment.
Percentages are based on the number of patients in the safety set in the specific treatment group.
*Eight patients who were randomised to the T&E ranibizumab alone group or the PRN ranibizumab group, supposed to be noted with laser treatment equal to 0, actually received laser treatment in the study eye. These patients were assigned to T&E ranibizumab+laser group in the safety analysis set, although laser was not considered as the study treatment because of the initial randomisation, and as such, these patients were not considered in the analysis of the number of study laser treatments. Because the laser treatment of these eight patients was not the randomised study medication, these cases were recorded in the concomitant medication dataset.
†Treatment visits do not include protocol-mandated intermediary visits.
‡Total number of scheduled treatment visits in this period over all patients in the treatment group.
§Interval after the visit with initial VA stability up to month 24.
¶Percentages are based on n, the number of patients in the safety set and with at least one treatment administered on or after the visit with initial VA stability in the specific treatment group.
BCVA, best-corrected visual acuity; PRN, pro re nata; T&E, treat-and-extend; VA, visual acuity.

over 70% patients in the T&E groups maintained their BCVA improvement with intertreatment intervals of ≥2 months after the visit with initial BCVA stability up to month 24. Similarly, data from the DRCR.net (protocol I),[8 9] READ-2[21] and the recent RELIGHT[16] studies indicate that extended monitoring intervals are possible without a negative impact on BCVA outcomes.

In RETAIN, combining laser with T&E ranibizumab did not provide additional improvements in BCVA outcomes and did not affect the number of injections needed, consistent with findings from the DRCR.net (protocol I)[8 9] and RESTORE studies.[4 6] The majority of patients in the T&E +laser group (77.8%) received a single laser treatment at baseline and did not require any additional through 24 months; the number of laser treatment in RETAIN was similar to that observed in protocol I.[8 9] The effects of laser may vary based on differing practices of laser administration among investigators. Findings in the T&E ranibizumab and PRN groups for efficacy and safety outcomes were consistent with those observed with the T&E ranibizumab with laser group in the study.

Patients with diabetes are at a higher risk of comorbidities and systemic complications.[22 23] However, no new safety risks were identified with ranibizumab 0.5 mg used in the RETAIN study. Overall, the incidence of ocular/non-ocular AEs was low and consistent with that observed in the previously reported clinical studies of ranibizumab in DMO.[4 6 8]

To our knowledge, RETAIN is the first prospective study designed to evaluate a T&E regimen in the management of DMO. The study was single-masked considering that the treating investigator would see the laser burns and patients with prior laser experience can distinguish true laser from sham laser treatments. The study protocol was conservative with respect to T&E to ensure patients do not lose vision. Overall, the inclusion and exclusion criteria of RETAIN were inclusive and re-treatment decisions and decisions on laser treatment were largely based on the investigator's judgement, reflecting a real-life clinical setting. The RETAIN study had certain limitations: (1) no central reading centre for CSFT measurement. (2) Re-treatment relied on VA loss, but the degree of loss was not defined and may thus vary by site, reflecting the clinical practice of the investigator. (3) Besides BCVA loss, changes in anatomical outcomes could also have been a consideration for re-treatment decisions. Examination of re-treatment visits in the PRN arm revealed that the VA loss from the previous visit was, on average, five letters and approximately 85% of re-treatments were accompanied by a loss in BCVA. In cases where treatment was administered without VA loss, the treatment decision may have been based on anatomical parameters, which may be a reflection of real-life clinical practice and is also consistent with the revised ranibizumab EU product label (2014)[24]. (4) Patients with previous stroke and transient ischaemic attack (high-risk patients) were excluded from the study. Finally, (5) the T&E regimen may have been too conservative, resulting in more injections than with PRN treatment, and its full potential with respect to reduction in number of visits and injections may still need to be determined.

In conclusion, the RETAIN study demonstrated that a ranibizumab T&E regimen is an appropriate alternative to a PRN regimen for management of DMO. This regimen allows for fewer clinic visits through extended intervals between treatments, thus providing the opportunity to reduce treatment burden and the potential to improve treatment compliance. Further studies using the T&E regimen with longer follow-up are required to explore the real-life implications using ranibizumab for treatment of patients with DMO. Reflecting this conclusion, the current ranibizumab EU product label (2014)[24] allows intertreatment intervals to be extended per individual patient case based on the treating physician's opinion and assessment of disease activity.

Author affiliations
[1]Department of Ophthalmology, Vista Klinik, Binningen, Switzerland
[2]Kantonsspital Baselland, Eye Clinic, Liestal, Switzerland
[3]University of Basel, Basel, Switzerland
[4]Centre d'Imagerie et Laser, Paris, France
[5]Royal Eye Hospital, Manchester, UK
[6]PTV Foundation, University of Rome "Tor Vergata", Rome, Italy
[7]Department of Ophthalmology, University Hospital, Hradec Králové, Czech Republic
[8]Novartis Pharma AG, Basel, Switzerland
[9]AIBILI, Coimbra, Portugal
[10]Coimbra Hospital and University Centre, Coimbra, Portugal
[11]Faculty of Medicine, University of Coimbra, Coimbra, Portugal

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Collaborators Group members are listed in online supplementary file S1.

Contributors Conception and design of the study: CP, VB, SP and AW. Analysis and interpretation: CP, FF, SM, FR, KH, JS, VB, SP, WJS, AW and JF. Critical revision of the article: CP, FF, KH, FR, SM, WJS, AW, SP and JF. Final approval of the article: CP, FF, SM, FR, KH, JS, VB, SP, WJS, AW and JF. Data collection: VB, SP, WJS and AW.

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Competing interests Professor CP receives grants and fees from Alcon, Allergan, Bayer and Novartis outside of this work; FF reports personal fees from Novartis, personal fees from Bayer, personal fees from Allergan, outside the submitted work; SM receives grant and personal fee from Novartis, Bayer, Allergan and personal fee from Alcon; FR reports non-financial support and other from Novartis, during the conduct of the study; grants, personal fees and non-financial support from Novartis, outside the submitted work; JS reports personal fees from Novartis s.r.o., personal fees from Bayer s.r.o., personal fees from Ewopharma, spol. s r.o., outside the submitted work; VB, SP, WJS and AW report other from Novartis Pharma AG, during the conduct of the study; other from Novartis Pharma AG, outside the submitted work; JF reports grants from AIBILI, during the conduct of the study; personal fees from Allergan and Kemin, non-financial support from Alcon, Alimera and Bayer, outside the submitted work; KH reports fees for lectures and travel support from Bayer, Switzerland and Allergan, and fees for lectures from Novartis and Roche.

Patient consent Obtained.

Ethics approval The study adhered to the tenets of the Declaration of Helsinki, the International Conference on Harmonization and Good Clinical Practice guidelines. The protocol and amendments were approved by the Independent Ethics Committee or Institutional Review Board for each participating centre.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement Full trial protocol can be assessed at: http://www.novctrd.com/ctrdWebApp/clinicaltrialrepository/displayFile.do?trialResult=11543

REFERENCES

1 Klein R, Klein BE, Moss SE. Visual impairment in diabetes. *Ophthalmology* 1984;91:1–9.
2 Antonetti DA, Klein R, Gardner TW. Diabetic retinopathy. *N Engl J Med* 2012;366:1227–39.
3 Chen E, Looman M, Laouri M, *et al*. Burden of illness of diabetic macular edema: literature review. *Curr Med Res Opin* 2010;26:1587–97.
4 Mitchell P, Bandello F, Schmidt-Erfurth U, *et al*. The RESTORE study: ranibizumab monotherapy or combined with laser versus laser monotherapy for diabetic macular edema. *Ophthalmology* 2011;118:615–25.
5 Lang GE, Berta A, Eldem BM, *et al*. Two-year safety and efficacy of ranibizumab 0.5 mg in diabetic macular edema: Interim analysis of the RESTORE Extension Study. *Ophthalmology* 2013;120:2004–12.
6 Schmidt-Erfurth U, Lang GE, Holz FG, *et al*. Three-year outcomes of individualized ranibizumab treatment in patients with diabetic macular edema: the RESTORE Extension study. *Ophthalmology* 2014;121:1045–53.
7 Elman MJ, Bressler NM, Qin H, *et al*, Diabetic Retinopathy Clinical Research Network. Expanded 2-year follow-up of ranibizumab plus prompt or deferred laser or triamcinolone plus prompt laser for diabetic macular edema. *Ophthalmology* 2011;118:609–14.
8 Elman MJ, Qin H, Aiello LP, *et al*, Diabetic Retinopathy Clinical Research Network. Intravitreal ranibizumab for diabetic macular edema with prompt versus deferred laser treatment: three-year randomized trial results. *Ophthalmology* 2012;119:2312–18.
9 Elman MJ, Ayala A, Bressler NM, *et al*. Intravitreal ranibizumab for diabetic macular edema with prompt versus deferred laser treatment: 5-year randomized trial results. *Ophthalmology* 2015;122:375–81.
10 Spaide RF. Ranibizumab according to need: a treatment for age-related macular degeneration. *Am J Ophthalmol* 2007;143:679–80.
11 Gupta OP, Shienbaum G, Patel AH, *et al*. A treat and extend regimen using ranibizumab for neovascular age-related macular degeneration clinical and economic impact. *Ophthalmology* 2010;117:2134–40.
12 Abedi F, Wickremasinghe S, Islam AF, *et al*. Anti-VEGF treatment in neovascular age-related macular degeneration: a treat-and-extend protocol over 2 years. *Retina* 2014;34:1531–8.

13 Rayess N, Houston SK III, Gupta OP, *et al*. Treatment outcomes after 3 years in neovascular age-related macular degeneration using a treat-and-extend regimen. *Am J Ophthalmol* 2015;159:3–8.
14 Berg K, Pedersen TR, Sandvik L, *et al*. Comparison of ranibizumab and bevacizumab for neovascular age-related macular degeneration according to LUCAS treat-and-extend protocol. *Ophthalmology* 2015;122:146–52.
15 Oubraham H, Cohen SY, Samimi S, *et al*. Inject and extend dosing versus dosing as needed: a comparative retrospective study of ranibizumab in exudative age-related macular degeneration. *Retina* 2011;31:26–30.
16 Pearce I, Banerjee S, Burton BJ, *et al*. Ranibizumab 0.5 mg for Diabetic Macular Edema with Bimonthly Monitoring after a Phase of Initial Treatment: 18-Month, Multicenter, Phase IIIB RELIGHT Study. *Ophthalmology* 2015;122:1811–19.
17 European Medicines Agency. Lucentis: EPAR-Product Information. EMEA/H/C/000715-IB/0032. Updated 20 July, 2012. http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/000715/WC500043546.pdf (accessed 15 May 2015).
18 Bhagat N, Grigorian RA, Tutela A, *et al*. Diabetic Macular Edema: pathogenesis and treatment. *Surv Ophthalmol* 2009;54:1–32.
19 Bretz F, Maurer W, Brannath W, *et al*. A graphical approach to sequentially rejective multiple test procedures. *Stat Med* 2009;28:586–604.
20 Toalster N, Russell M, Ng P. A 12-month prospective trial of inject and extend regimen for ranibizumab treatment of age-related macular degeneration. *Retina* 2013;33:1351–8.
21 Do DV, Nguyen QD, Khwaja AA, *et al*, for the READ-2 Study Group. Ranibizumab for oedema of the macula in diabetes study: 3-year outcomes and the need for prolonged frequent treatment. *JAMA Ophthalmol* 2013;131:139–45.
22 Klein R, Klein BE, Moss SE, *et al*. The Wisconsin epidemiologic study of diabetic retinopathy. IV. Diabetic macular edema. *Ophthalmology* 1984;91:1464–74.
23 Mercer BN, Morais S, Cubbon RM, *et al*. Diabetes mellitus and the heart. *Int J Clin Pract* 2012;66:640–7.
24 European Medicines Agency. Summary of Product Characteristics. Lucentis 10 mg/ml solution for injection. Novartis. 2013. http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/000715/WC500043546.pdf http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/000715/WC500043546.pdf (accessed 15 May 2015).

Supplementary File S1: RETAIN investigators

List of primary investigators involved in patient recruitment

Belgium (2 centres)

Edward de Sutter and Joachim Van Calster

Czech Republic (5 centres)

Bohdana Kalvodova, Jan Ernest, Jan Studnicka, Jiri Rehak and Hana Fidranska

France (9 centres)

Pascale Massin, Franck Fajnkuchen, Maddalena Quaranta El Maftouhi, Catherine Creuzot-Garcher, Michel Weber, Pierre Labalette, Pierre-Yves Robert, Jean François Korobelnik and Stéphanie Baillif

Greece (3 centres)

Ioannis Datseris, Miltiadis Tsilimbaris and Fotis Topouzis

Hungary (4 centres)

András Seres, András Papp, András Berta and Norbert Pesztenléhrer

Italy (6 centres)

Giovanni Staurenghi, Ugo Menchini, Francesco Maria Bandello, Francesco Viola, Federico Ricci and Monica Varano

Ireland (2 centres)

Marie Hickey-Dwyer and Mark Cahill

Netherlands (5 centres)

R.O. Schlingemann, Yvonne De Jong-Hesse, Carel Hoyng, G. Dijkman and Jose P. Martinez Ciriano

Poland (4 centres)

Edward Wylegala, Marta Misiuk-Hojlo, Tomasz Zarnowski and Iwona Grabska-Liberek

Portugal (3 centres)

Joao Figueira, Miguel Marques and Angelina Meireles

Spain (8 centres)

Francisco Gómez Ulla, Lluis Arias Barquet, Juan Donate López, Jose Ruiz Moreno, Francisco Cabrera Lopez, Carlos Hernando, Maria López Gálvez and Amparo Navea Tejerina

Switzerland (4 centres)

Justus Garweg, Christian Prünte, Sebastian Wolf and Stephan Michels

United Kingdom (10 centres)

Clare Bailey, Christopher S Brand, Rajen Gupta, Andrew Lotery, Martin McKibbin, Geetha Menon, John Olson, Sajjad Mahmood, Deepali Varma and Niro Narendran

Patient enrolment summary by country

Country	**Total number of patients enrolled**
Belgium	4
Czech Republic	39
France	42
Greece	27
Hungary	28
Ireland	9
Italy	53
Netherlands	28
Poland	18
Portugal	12
Spain	40
Switzerland	25
United Kingdom	48
Total	**373**

Supplementary File S2: Exclusion criteria

Patient compliance/Administrative

1. Women of childbearing potential, defined as all women physiologically capable of becoming pregnant including women whose career, lifestyle, or sexual orientation precludes intercourse with a male partner and women whose partners have been sterilised by vasectomy or other means, unless they are using two birth control methods. The two methods can be a double barrier method or a barrier method plus a hormonal method. Adequate barrier methods of contraception include: diaphragm, condom (by the partner), intrauterine device (copper or hormonal), sponge or spermicide. Hormonal contraceptives include any marketed contraceptive agent that includes an oestrogen and/or a progestational agent.

2. Pregnant or nursing (lactating) women, where pregnancy is defined as the state of a female after conception and until the termination of gestation, confirmed by a positive serum pregnancy test (human chorionic gonadotropin >5 mIU/mL).

3. Inability to comply with study procedures.

Ocular medical history

4. Active intraocular inflammation (grade trace or above) in either eye at enrolment.

5. Any active infection (e.g. conjunctivitis, keratitis, scleritis, uveitis or endophthalmitis) in either eye at the time of enrolment.

6. History of uveitis in either eye at any time.

7. Structural damage within 0.5 disc diameter of the centre of the macular in the study eye likely to preclude improvement in visual acuity following the resolution of macular oedema, including atrophy of the retinal pigment epithelium, subretinal fibrosis, laser scar(s), epiretinal membrane involving fovea or organised hard exudate plaques.

8. Patients with both, a best-corrected visual acuity (BCVA) score of >73 letters and a central subfield thickness (CSFT) of <300 μm in the study eye.

9. Uncontrolled glaucoma in either eye at screening (intraocular pressure (IOP) >24 mmHg on medication or according to investigator's judgment).

10. Neovascularisation of the iris in either eye.

11. Evidence of vitreomacular traction in the study eye.

12. History of retinal detachment, retinal tear or macular hole in the study eye.

13. Active proliferative diabetic retinopathy in the study eye, i.e. any neovascularisation that progressed within 6 months prior to randomisation.

14. Patients who are monocular or have a BCVA score in the nonstudy eye (fellow eye) ≤24 letters (approximate Snellen equivalent of 20/320) at Visit 1.

Prior ocular treatments

15. Any intraocular surgery in the study eye within 3 months prior to randomisation.

16. History of vitrectomy in study eye regardless of time prior to randomisation.

17. Planned medical or surgical intervention during the 24-month study period.

18. Panretinal laser photocoagulation in the study eye within 6 months prior to randomisation.

19. Focal/grid laser photocoagulation in the study eye within 3 months prior to randomisation.

20. Treatment with antiangiogenic drugs in either eye (pegaptanib sodium, anecortave acetate, bevacizumab, ranibizumab or VEGF-Trap) within 3 months prior to randomisation.

21. Use of other investigational drugs at the time of enrolment or within 3 months or 5 half-lives from enrolment, whichever is longer.

22. History of intravitreal corticosteroid treatment in phakic study eye.

23. Intravitreal corticosteroids in postcataract surgery study eye (aphakic or pseudophakic, without damaged posterior capsule) within 3 months prior to randomisation.

24. Ocular conditions in the study eye that require chronic concomitant therapy with topical ocular corticosteroids.

25. Intraocular implants except for lenses.

Systemic conditions or treatments

26. History of stroke within 6 months prior to enrolment.

27. Renal failure requiring dialysis or renal transplant or renal insufficiency with creatinine levels >2.0 mg/dL at screening.

28. Untreated diabetes mellitus.

29. Blood pressure systolic >160 mmHg or diastolic >100 mmHg at screening and/or randomisation.

30. Untreated hypertension or change in antihypertensive treatment within 3 months preceding randomisation.

31. Conditions that require chronic concomitant therapy with systemically administered corticosteroids.

32. Current use of or likely need for systemic medications known to be toxic to the lens, retina or optic nerve, including deferoxamine, chloroquine/hydroxychloroquine (Plaquenil), tamoxifen, phenothiazines and ethambutol.

33. Known hypersensitivity to fluorescein or ranibizumab or any component thereof or drugs of similar chemical classes.

34. Any type of advanced, severe or unstable disease or its treatment that may interfere with primary and/or secondary variable evaluations including any medical condition that could be expected to progress, recur or change to such an extent that it may bias the assessment of the clinical status of the patient to a significant degree or put the patient at special risk.

Note: Exclusion criteria in RETAIN were similar to the RESTORE and REVEAL studies of ranibizumab in patients with diabetic macular oedema (DME) with respect to systemic conditions or treatments.

Supplementary File S3: Randomisation sequence, allocation and masking

The randomisation numbers were generated using the following procedure to ensure that treatment assignment was unbiased and concealed from patients and masked investigator staff. RETAIN was designed as a single masked study. It was impossible to double-mask the laser treatment because the Treating Investigator could potentially identify laser burns on fundus images, and those patients with previous experience of laser treatment would have been able to differentiate between sham and active laser treatments. Thus, in order to provide a reasonable degree of masking, the study was observer-masked and for fulfilment of the masking requirements each site required a masked 'Evaluating Investigator' and an unmasked 'Treating Investigator'. The evaluating investigator (masked to the treatment assignment) performed BCVA and other study efficacy assessments but did not perform postinjection IOP measurements and judged the presence or absence of BCVA stability and the presence or absence of DME disease activity/recurrence. He/she provided this information to the treating investigator(s). The treating investigator(s) (unmasked to the treatment assignment) received treatment allocation cards and administered study treatment (ranibizumab injections or laser treatment) based on the judgment of the evaluating investigator regarding BCVA stability and DME activity/recurrence and according to treatment schedule (i.e. group) a given patient had been randomised to as per treatment allocation cards. The treating investigator(s) was not involved in any study efficacy evaluations and did not divulge details of the treatment assignment to anyone.

Each patient was uniquely identified in the study by a combination of his/her centre number and patient number. The centre number was assigned by Novartis to the investigative site. Upon signing the informed consent, the patient was assigned a patient number by the BCVA assessing investigator. At Visit 2, the treating investigator randomised patients who fulfilled the inclusion/exclusion criteria using the sealed treatment allocation cards supplied by Novartis. The investigator was required to maintain an accurate record of the shipment and dispensing of study drug in a drug accountability ledger. Monitoring of drug accountability was performed by the field monitor during site visits and at the completion of the trial. Novartis drug supply management provided sufficient supplies of ranibizumab 0.5 mg for treatment to each study site.

Supplementary File S4: Treat-and-extend (T&E) regimen and study design description

1) Ranibizumab treatment–all three groups. For all three treatment groups, there was an initial period of monthly treatment until the vision was stabilised, and after which the intertreatment intervals could be extended to 2 months or greater. Patients went back to monthly injections upon experiencing any visual acuity (VA) loss. As the study was a first time assessment of the treat-and-extend (T&E) concept in DME, patients in all three treatment groups were required to attend monitoring visits.

(i) All treatment groups–monthly treatment until reaching stability

Patients received monthly injections of ranibizumab 0.5 mg for 3 consecutive months until VA was stable, i.e. neither improvement nor deterioration for three consecutive monthly visits observed while on treatment. At the visit at which BCVA stabilisation was confirmed, the patient did not receive any treatment (no ranibizumab or laser) and followed the next stage in the T&E design.

(ii) After stability had been confirmed

a) Ranibizumab T&E with or without laser. For these two groups, there were two types of visits: (i) T&E visits which were scheduled for mandatory treatment and included a decision point for potential reinitiating of monthly injections and (ii) Intermediary visits for patients to remain masked to their treatment assignment and to collect efficacy and safety data as routine in clinical practice. At these visits, no treatment was administered or no decisions for study treatment were made.

The first T&E visit followed 1 month after the visit at which stabilisation was confirmed, i.e. for a patient who had stable VA (for an ideal case, at the end of the third month in the study, i.e. end of Month 2) the first T&E visit with mandatory injection was 2 months after the end of monthly injection phase (for an ideal case, Month 4). If there was no loss of BCVA that was attributable at this visit, the next T&E visit was scheduled 3 months later (for an ideal case, Month 7). Hence, the treatment-free interval was increased by an additional 1 month and the following two visits were intermediary visits. This schedule for T&E visits persisted as long as stabilisation was confirmed at the subsequent T&E visits. The study design did not permit treatment intervals of >3 months.

The retreatment criterion was based on VA stability criteria. Though not defined by the study protocol, Optical Coherence Tomography (OCT) may well have been used to guide/aid the retreatment decisions by certain centres, thereby providing an opportunity for a proactive approach at detecting the early signs of disease activity before the actual vision loss.

b) Ranibizumab 0.5 mg PRN. Patients in this treatment group continued on the ranibizumab pro re nata (PRN) treatment regimen as per the European Summary of Product Characteristics (EU SmPC) 2011. Thus, each monitoring visit for PRN entailed assessment of BCVA stability and a decision about potential reinitiation of monthly injections if VA was not stable over the last 3 consecutive monthly visits.

Laser treatment in the T&E+laser group

In the T&E+laser group, patients received laser treatment on Day 1, following which treatment could be readministered based on the early treatment diabetic retinopathy study (ETDRS) guidelines. Treatment was at the discretion of the investigator, and the minimum recommended interval between laser treatments was 3 months. If the investigator deemed the patient required both ranibizumab and laser treatments on the same day, then laser was administered ≥30 minutes prior to the ranibizumab injection.

Retreatment criteria

The retreatment criterion was as defined in the EU SmPC 2011, i.e. treatment is resumed if a patient's vision worsens due to their disease. Accordingly, as soon as at a T&E visit BCVA loss due to recurrent DME disease activity was observed, in comparison to the last T&E visit or to the last visit of monthly injections, whichever occurred later, the patient re-entered the monthly injection phase. The treatment given at this visit was considered the first treatment of the newly entered monthly injection phase. The patient subsequently received monthly treatment until BCVA was stable again. If two consecutive attempts to extend the treatment-free interval in T&E were unsuccessful i.e. there was a loss of BCVA at the end of each extension and the patient re-entered monthly injection phase twice, the future maximum treatment-free period was shortened by 1 month. Thus, patients could fall back to mandatory monthly treatment by this mechanism. Further, the respective intervals between treatments could have been too long to maintain stable BCVA in a given patient. In the PRN regimen, each visit following VA

stabilisation was a potential treatment visit, with the treatment being administered based upon assessment of the patient's vision at that visit.

Individual patients may have received retreatment based on anatomical findings (OCT imaging) in the absence of VA loss, as would have been the standard procedure in routine clinical practice. This practise may help to treat the disease at an earlier stage.

Supplementary File S5: Statistical analysis details

Hypotheses of primary objective for noninferiority and superiority were tested using a sequentially rejective multiple testing procedure that protects the multiple one-sided alpha level of 0.025. In order to protect the multiple one-sided alpha level of 0.025, a sequentially rejective testing procedure was established.

Hypotheses related to noninferiority were:

•Null hypotheses: H_{01}: μIII - μI >4 letters and H_{02}: μIII - μII >4 letters

•Alternative hypotheses: H_{11}: μIII - μI ≤4 letters and H_{12}: μIII - μI ≤4 letters,

where μI, μII and μIII were defined as the primary efficacy variable mean of the average change from baseline to Month 1 through Month 12 in BCVA obtained from all treatment groups (0.5 mg ranibizumab T&E+laser, 0.5 mg ranibizumab T&E alone and 0.5 mg ranibizumab PRN).

Hypotheses related to superiority were:

•Null hypotheses: H_{03}: μIII - μI ≥0 letters and H_{04}: μIII - μII ≥0 letters

•Alternative hypotheses: H_{13}: μIII - μI < 0 letters and H_{14}: μIII - μII <0 letters

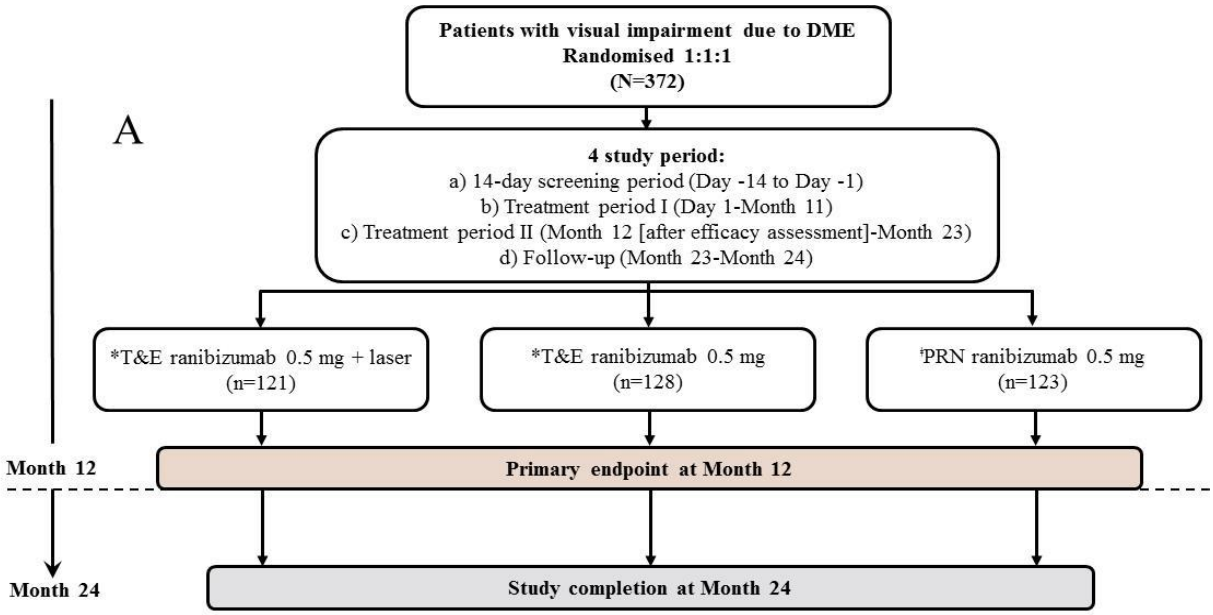
The noninferiority margin of 4 letters was used during testing (indicated by the U.S. Food and Drug Administration in regulatory discussions of Visudyne studies in 2008). The primary analysis was carried out after patients had completed the Month 12 visit using the full analysis set (FAS) which consisted of all randomised patients who received at least one application of the study treatment (ranibizumab or laser), and had at least one postbaseline assessment for BCVA in the study eye. The mean value imputation/last observation carried forward (MV/LOCF) approach was employed to compensate for the missing data. Following the intent-to-treat principle, patients were analysed according to the treatment assigned at randomisation. All analyses for the secondary efficacy variables were carried out on untransformed data. For analyses based on proportions, corresponding 95% confidence intervals (CIs) for each treatment group were calculated using exact methods, and the 95% CI of the difference in proportions between treatment groups was computed based on the normal approximation. For continuous variables, descriptive statistics by scheduled visit were provided for absolute values and changes from baseline. Variables expressed as proportion of patients were also presented by scheduled visit with number and percentage of patients. The statistical analysis was performed by Parexel personnel according to the statistical analysis plan.

Supplementary File S6: Levels of visual impairment– World Health Organization (WHO) International Classification of Functioning, Disability and Health (ICF), Switzerland

Classification		**Levels of Visual Impairment**	**Additional Descriptors That may be Encountered**
Legal	WHO	Visual Acuity (VA) and/or Visual Field (VF) Limitation (whichever is worse)	
	(NEAR-) NORMAL VISION	RANGE OF NORMAL VISION 20/10 20/13 20/16 20/20 20/25 2.0 1.6 1.25 1.0 0.8	
		NEAR-NORMAL VISION 20/28 20/30 20/40 20/50 20/60 0.7 0.6 0.5 0.4 0.4	
	LOW VISION	MODERATE VISUAL IMPAIRMENT 20/70 20/80 20/100 20/125 20/160 0.29 0.25 0.20 0.16 0.12	Moderate low vision
		SEVERE VISUAL IMPAIRMENT 20/200 20/250 20/320 20/400 0.10 0.08 0.06 0.05 VF 20 degrees or less	Severe low vision, 'Legal' blindness
	BLINDNESS (WHO)	PROFOUND VISUAL IMPAIRMENT 20/500 20/630 20/800 20/1000 0.04 0.03 0.025 0.02 CF at: less than 3m (10 ft.) VF: 10 degrees or less	Profound low vision, Moderate blindness
	One or both eyes	NEAR-TOTAL VISUAL IMPAIRMENT VA: less than 0.02 (20/1000) CF at: 1m (3 ft.) or less HM: 5m (15 ft.) or less Light projection, light perception VF: 5 degrees or less	Severe blindness, Near-total blindness
		TOTAL VISUAL IMPAIRMENT No light perception (NLP)	Total blindness

CF = counts fingers (without designation of distance may be classified to profound impairment)
HM = hand motion (without designation of distance may be classified as near-total impairment)
VA = visual acuity (refers to best achievable acuity with correction)
VF = visual field (measurements refer to the largest field diameter for a 1/100 white test object)
Modified from the International Classification of Diseases, 9th rev. Clinical Modification

Supplementary Figure S1: RETAIN (A) study design and (B) treatment schedule



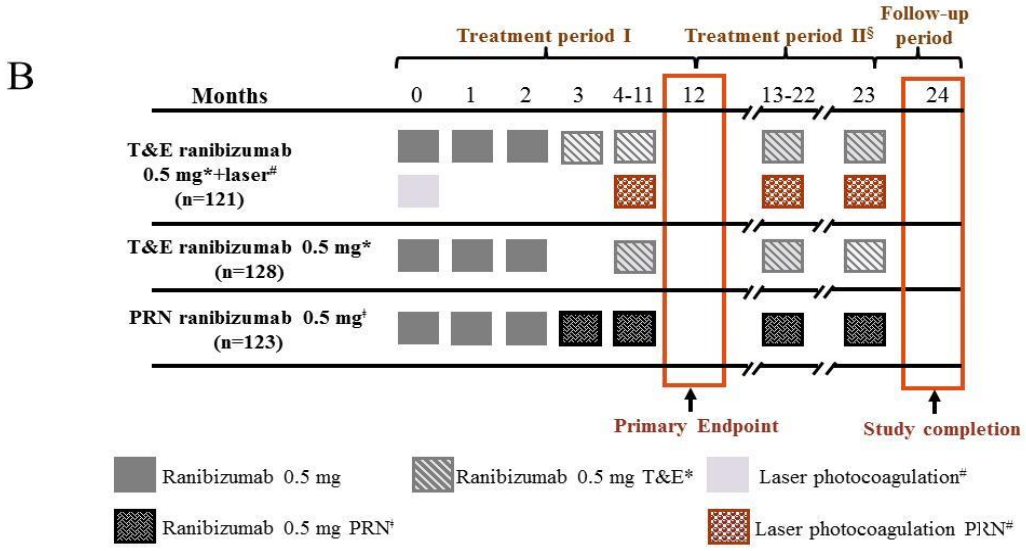
Note: Treatment was resumed if a patient's vision worsens due to their disease, as per the retreatment criterion defined in European SmPC

*Ranibizumab injection at baseline (T&E+laser: additional laser treatment at baseline and PRN subsequently according to ETDRS guidelines); monthly ranibizumab 0.5 mg treatment until BCVA stability, monthly monitoring, incremental extension in the inter-treatment interval by 1 month (maximum prolongation up to 3 months) at stable BCVA, when BCVA decreases due to DME at T&E visit resume monthly treatment until BCVA stability and re-enter the extension treatment phase

†Ranibizumab injection at baseline, monthly treatment until BCVA stability, monthly monitoring, when BCVA decreases due to DME resume monthly treatment until BCVA stability; ranibizumab given PRN

13 countries included Belgium, Czech Republic, France, Greece, Hungary, Ireland, Italy, the Netherlands, Poland, Portugal, Spain, Switzerland, and the United Kingdom

BCVA, best-corrected visual acuity; DME, diabetic macular oedema; ETDRS, early treatment diabetic retinopathy study; PRN, pro re nata; T&E, treat-and-extend



§After assessment of efficacy for BCVA stability

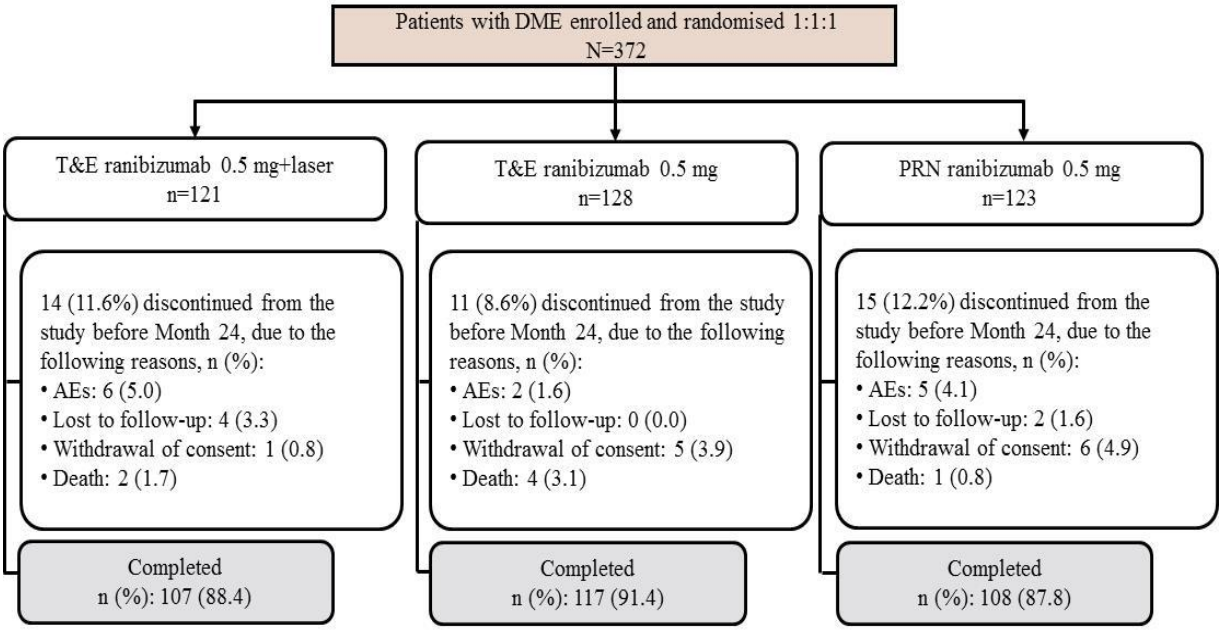
*Ranibizumab 0.5 mg T&E: monthly ranibizumab 0.5 mg administered until BCVA stability (no BCVA change for 3 consecutive monthly visits; phase-A), monthly monitoring, followed by incremental extension of treatment-free interval by 1 month, up to a maximum interval of 3 months, at stable VA (phase-B). If a decrease in VA is observed due to DME at the T&E visit, phase-A is resumed followed by phase-B

†Ranibizumab 0.5 mg PRN: monthly ranibizumab 0.5 mg administered until BCVA stability (no BCVA change for 3 consecutive monthly visits; phase-A), monthly monitoring; If BCVA decrease due to DME observed, monthly ranibizumab treatment resumed until VA stability (Phase A)

#Laser: Administered at baseline and thereafter at a minimum interval of 90 days, according to the ETDRS guidelines at investigator's discretion

BCVA, best-corrected visual acuity; DME, diabetic macular oedema; ETDRS, early treatment diabetic retinopathy study; PRN, pro re nata; T&E, treat-and-extend; VA, visual acuity

Supplementary Figure S2: Patient disposition flowchart



AE, adverse event; DME, diabetic macular oedema; PRN, pro re nata; T&E, treat-and-extend

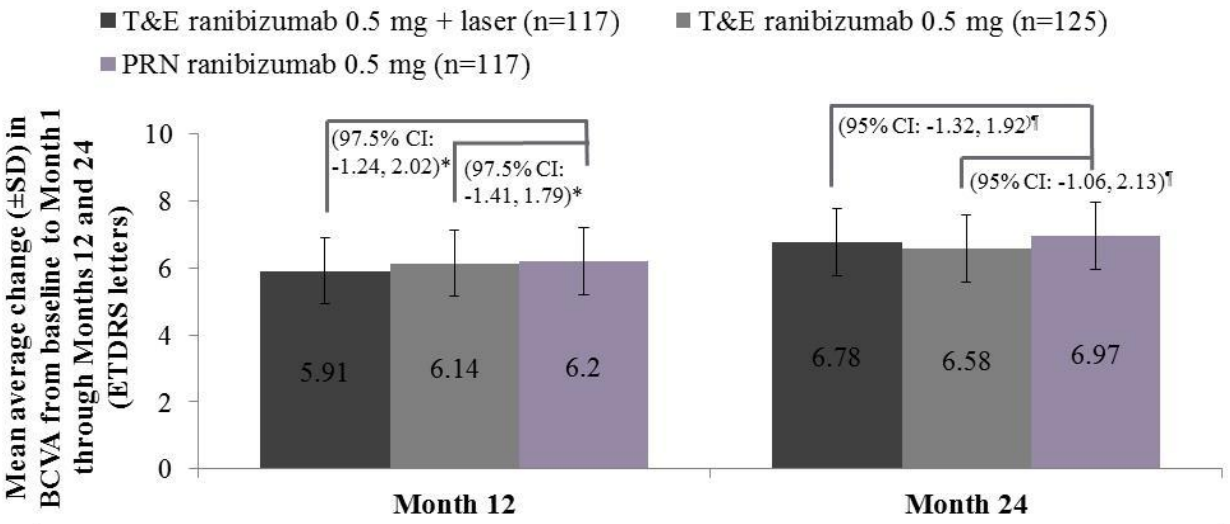
Analyses patient sets

Analysis sets	T&E ranibizumab 0.5 mg+laser n (%)	T&E ranibizumab 0.5 mg n (%)	PRN ranibizumab 0.5 mg n (%)	Total n (%)
Randomised set	121 (100)	128 (100)	123 (100)	372 (100)
Full analysis set	117 (96.7)	125 (97.7)	117 (95.1)	359 (96.5)
Safety set	126	126	118	370

Percentages are based on the total number of patients in the randomised set in the specific treatment group.
Safety set is based on actual treatment group; other populations are based on the randomised treatment group.
Number of patients in the safety set was lower than in the randomised set due to two patients who did not receive any study medication. Both patients were randomised but discontinued before any study drug was administered. There were four patients randomised to the PRN group and three patients randomised to T&E group who received laser treatment and were analysed in the safety set as part of the T&E+laser group. Also, two patients randomised to the T&E+laser group did not receive laser treatment and therefore were analysed in the safety set as part of the T&E group.
The randomised set comprised all randomised patients, i.e. those given a randomisation number.
The full analysis set consisted of all randomised patients who received at least one application of study treatment (ranibizumab or laser), and had at least one postbaseline efficacy assessment in the study eye.
The safety set consisted of all patients who received at least one administration of study treatment in the study eye and had at least one postbaseline safety assessment.
PRN, pro re nata; T&E, treat-and-extend

Note: The analyses presented in RETAIN were all performed with the FAS at Month 24. The two FAS analysed for Month 12 at the two time points (Months 12 and 24) were different due to the need to exclude 10 patients from the Month 24 analysis at a single site due to Good Clinical Practice deviations. The impact of this difference on the efficacy outcomes was negligible across groups (BCVA difference 0.03–0.1 letters, CSFT ~2%; range: 0.33–2.32). The safety data from these 10 patients were included in the analysis. The inclusion of patients in the safety set was determined by the actual treatment received, rather than the treatment group the patient was randomised to.

Supplementary Figure S3: The mean average change in best-corrected visual acuity (BCVA) from baseline to Month 1 through Months 12 (primary endpoint) and 24 (FAS–MV/LOCF)



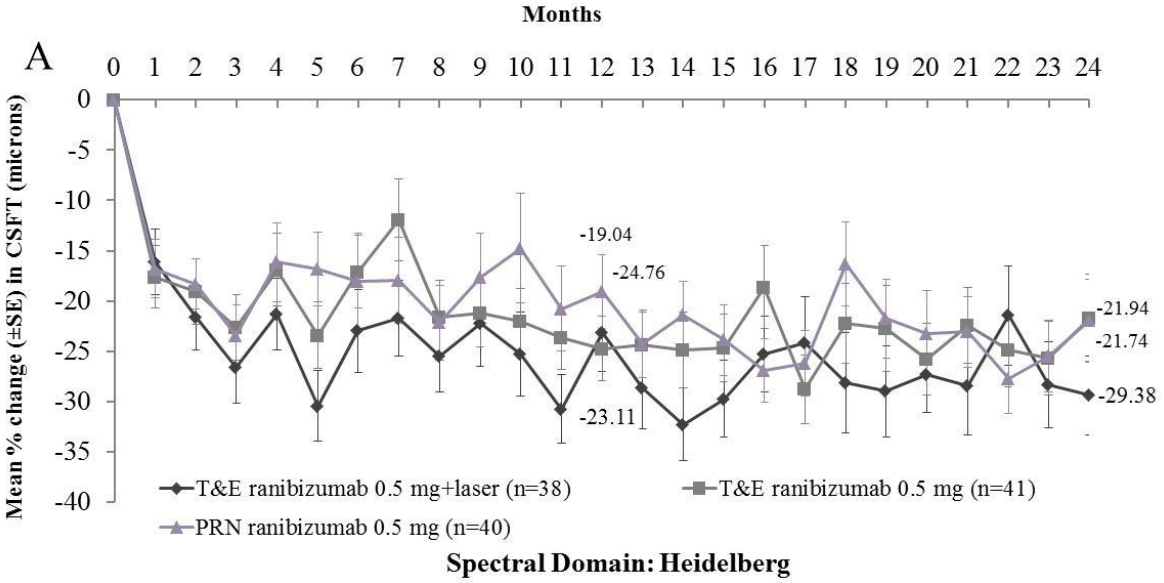
Non-inferiority of each of the T&E ranibizumab groups to the PRN ranibizumab group was established applying the CMH test with both nominal p-values <0.0001 which is less than <0.0125 as required for statistical significance at the multiple one-sided 2.5% significance level

¶Upper limits of the 97.5% CIs for the differences were <4 letters for both comparisons; subsequent assessment did not confirm the superiority of both T&E regimens over the PRN regimen. Assessments of superiority of the T&E ranibizumab groups over PRN ranibizumab group using nominal one-sided p values for untransformed CMH tests showed that the comparisons of T&E ranibizumab+laser vs PRN ranibizumab (p=0.7064) and T&E ranibizumab vs PRN ranibizumab (p=0.6052), respectively, were statistically non-significant exceeding the critical level of 0.0125 for both comparisons

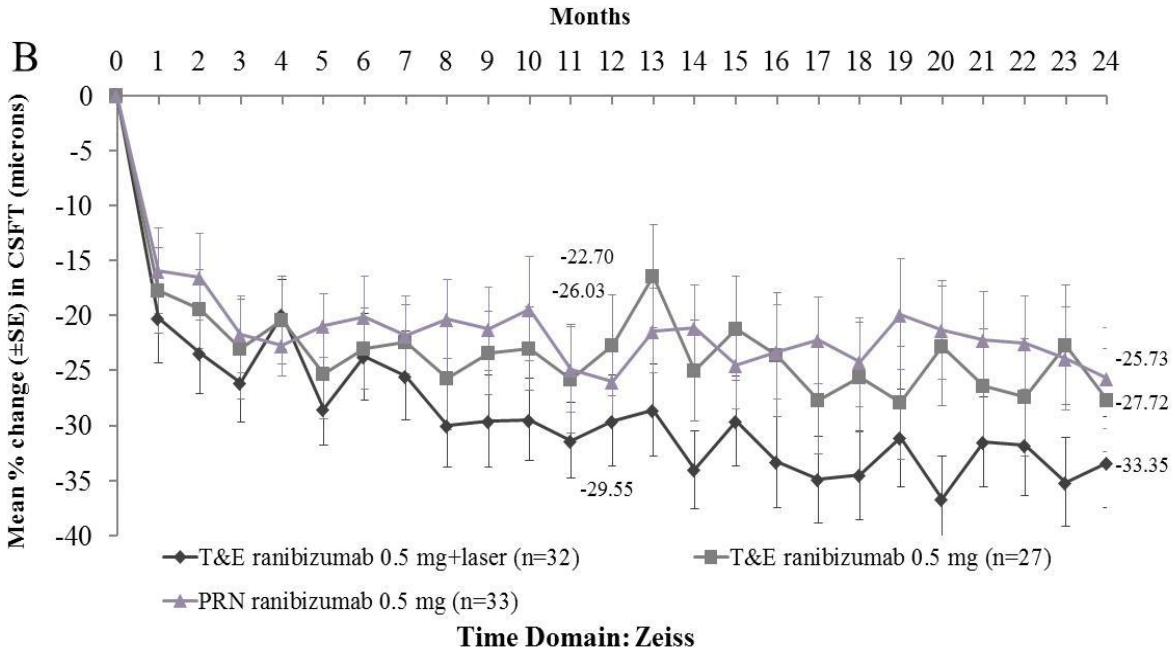
*ANOVA untransformed comparison of the LS mean changes; Full analysis set (MV/LOCF): consisted of all randomized patients who received at least one application of study treatment (ranibizumab or laser), and had at least one post baseline efficacy assessment in the study eye.); Stratified analysis includes baseline VA (≤60 letters, >60 letters and ≤73 letters, >73 letters) as factor

BCVA, best-corrected visual acuity; CI, confidence interval; CMH, Cochran-Mantel-Haenszel; ETDRS, early treatment diabetic retinopathy study; FAS, full analysis set; MV/LOCF, mean value imputation/last observation carried forward; PRN, pro re nata; SD, standard deviation; T&E, treat-and-extend; VA, visual acuity

Supplementary Figure S4: The mean percentage change in central subfield thickness (CSFT) from baseline by Optical Coherence Tomography (OCT) machine type—(A) Heidelberg and (B) Zeiss (FAS–MV/LOCF)



Full analysis set consisted of all randomized patients who received at least one application of study treatment (ranibizumab or laser), and had at least one post baseline efficacy assessment in the study eye)
CSFT, central subfield thickness; FAS, full analysis set; MV/LOCF, mean value imputation/last observation carried forward; OCT, optical coherence tomography; PRN, pro re nata; SE, standard error; T&E, treat-and-extend



Full analysis set consisted of all randomized patients who received at least one application of study treatment (ranibizumab or laser), and had at least one post baseline efficacy assessment in the study eye)
CSFT, central subfield thickness; FAS, full analysis set; MV/LOCF, mean value imputation/last observation carried forward; OCT, optical coherence tomography; PRN, pro re nata; SE, standard error; T&E, treat-and-extend

Supplementary Table S1: Most frequent ocular and nonocular adverse events (at least 2% in any group) by preferred term (safety set)

Primary system organ class Preferred term, n (%)	T&E ranibizumab 0.5 mg+laser n=126	T&E ranibizumab 0.5 mg n=126	PRN ranibizumab 0.5 mg n=118
Any ocular AE, Total	58 (46.0)	63 (50.0)	46 (39.0)
Cataract*†	5 (4.0)	7 (5.6)	7 (5.9)
Conjunctival haemorrhage*	5 (4.0)	11 (8.7)	6 (5.1)
Dry eye	6 (4.8)	3 (2.4)	4 (3.4)
Vitreous floaters†	0 (0.0)	1 (0.8)	4 (3.4)
Eye pain*	8 (6.3)	7 (5.6)	3 (2.5)
Ocular hypertension*†	7 (5.6)	2 (1.6)	3 (2.5)
Eye irritation	2 (1.6)	4 (3.2)	2 (1.7)
Macular oedema	2 (1.6)	3 (2.4)	2 (1.7)
Diabetic retinal oedema	4 (3.2)	2 (1.6)	1 (0.8)
Glaucoma*†	3 (2.4)	1 (0.8)	1 (0.8)
Conjunctivitis	6 (4.8)	2 (1.6)	0 (0.0)
Keratitis	1 (0.8)	3 (2.4)	0 (0.0)
Lacrimation increased*	1 (0.8)	4 (3.2)	0 (0.0)
Macular fibrosis	3 (2.4)	0 (0.0)	0 (0.0)
Retinal haemorrhage*	2 (1.6)	3 (2.4)	0 (0.0)
Vitreous haemorrhage*†	8 (6.3)	2 (1.6)	0 (0.0)
IOP increased*†	2 (1.6)	7 (5.6)	5 (4.2)
Any nonocular AE, Total	97 (77.0)	99 (78.6)	83 (70.3)
Blood and lymphatic system disorders	5 (4.0)	6 (4.8)	7 (5.9)
Anaemia	4 (3.2)	5 (4.0)	5 (4.2)
Gastrointestinal disorders	22 (17.5)	18 (14.3)	13 (11.0)
Diarrhoea	4 (3.2)	4 (3.2)	7 (5.9)
General disorders and administrative site conditions	14 (11.1)	13 (10.3)	14 (11.9)
Oedema peripheral	7 (5.6)	5 (4.0)	3 (2.5)
Infections and infestations	56 (44.4)	54 (42.9)	42 (35.6)
Influenza	9 (7.1)	10 (7.9)	8 (6.8)
Nasopharyngitis	11 (8.7)	10 (7.9)	8 (6.8)
Cystitis	2 (1.6)	2 (1.6)	6 (5.1)
Bronchitis	11 (8.7)	1 (0.8)	5 (4.2)
Urinary tract infection	9 (7.1)	11 (8.7)	5 (4.2)
Investigations	11 (8.7)	21 (16.7)	14 (11.9)
Blood urine present	2 (1.6)	3 (2.4)	5 (4.2)
Musculoskeletal and connective tissue disorders	21 (16.7)	23 (18.3)	13 (11.0)
Back pain	8 (6.3)	10 (7.9)	3 (2.5)
Vascular disorders	28 (22.2)	23 (18.3)	18 (15.3)
Hypertension	20 (15.9)	18 (14.3)	8 (6.8)

Safety set consisted of all patients who received at least one active application of study treatment and had at least one postbaseline safety assessment

*suspected to be related to ocular injection

†suspected to be related to treatment

A patient with multiple occurrences of a preferred term is counted only once in the preferred term row

A patient with multiple adverse events within a primary system organ class is counted only once in the total row

Percentages are based on the number of patients in the safety set in the specific treatment group

AE, adverse event; IOP, intraocular pressure; PRN, pro re nata; T&E, treat-and-extend

Supplementary Table S2: Ocular (study eye) and nonocular adverse events leading to permanent study treatment discontinuation, regardless of study treatment relationship, by primary system organ class and preferred term (safety set)

Primary system organ class Preferred term, n (%)	T&E ranibizumab 0.5 mg+laser n=126	T&E ranibizumab 0.5 mg n=126	PRN ranibizumab 0.5 mg n=118
Ocular AEs			
Any primary system organ class	1 (0.8)	0 (0.0)	0 (0.0)
Eye disorders	1 (0.8)	0 (0.0)	0 (0.0)
Macular fibrosis	1 (0.8)	0 (0.0)	0 (0.0)
Nonocular AEs			
Any primary system organ class	9 (7.1)	6 (4.8)	5 (4.2)
Cardiac disorders	1 (0.8)	3 (2.4)	0 (0.0)
Acute myocardial infarction	0 (0.0)	1 (0.8)	0 (0.0)
Arrhythmia	0 (0.0)	1 (0.8)	0 (0.0)
Bradycardia	0 (0.0)	1 (0.8)	0 (0.0)
Coronary artery disease	0 (0.0)	1 (0.8)	0 (0.0)
Myocardial infarction	1 (0.8)	1 (0.8)	0 (0.0)
Myocardial ischemia	0 (0.0)	1 (0.8)	0 (0.0)
Metabolism and nutrition disorders	1 (0.8)	0 (0.0)	0 (0.0)
Dehydration	1 (0.8)	0 (0.0)	0 (0.0)
Musculoskeletal and connective tissue disorders	1 (0.8)	0 (0.0)	1 (0.8)
Pain in extremity	0 (0.0)	0 (0.0)	1 (0.8)
Intervertebral disc protrusion	1 (0.8)	0 (0.0)	0 (0.0)
Myalgia	1 (0.8)	0 (0.0)	0 (0.0)
Spinal osteoarthritis	1 (0.8)	0 (0.0)	0 (0.0)
Neoplasms benign, malignant and unspecified (incl. cysts and polyps)	2 (1.6)	0 (0.0)	2 (1.7)
Hepatic cancer	0 (0.0)	0 (0.0)	1 (0.8)
Pancreatic carcinoma	0 (0.0)	0 (0.0)	1 (0.8)
Lung adenocarcinoma	1 (0.8)	0 (0.0)	0 (0.0)
Metastases to peritoneum	1 (0.8)	0 (0.0)	0 (0.0)
Nervous system disorders	1 (0.8)	2 (1.6)	1 (0.8)
Cerebrovascular accident	1 (0.8)	1 (0.8)	1 (0.8)
Transient ischemic attack	0 (0.0)	1 (0.8)	0 (0.0)
Renal and urinary disorders	1 (0.8)	0 (0.0)	1 (0.8)
Haematuria	0 (0.0)	0 (0.0)	1 (0.8)
Renal mass	1 (0.8)	0 (0.0)	0 (0.0)
Respiratory, thoracic and mediastinal disorder	1 (0.8)	1 (0.8)	0 (0.0)
Acute respiratory failure	0 (0.0)	1 (0.8)	0 (0.0)
Pulmonary embolism	1 (0.8)	0 (0.0)	0 (0.0)
Vascular disorders	1 (0.8)	0 (0.0)	0 (0.0)
Hypertension	1 (0.8)	0 (0.0)	0 (0.0)

Safety set consisted of all patients who received at least one active application of study treatment and had at least one post-baseline safety assessment
Percentages are based on the number of patients in the Safety set in the specific treatment group
AEs, adverse events; PRN, pro re nata; T&E, treat-and-extend

Supplementary Table S3: Key SAEs by primary system organ class and preferred term (safety set)

Primary system organ class Preferred term	T&E ranibizumab 0.5 mg+laser n=126	T&E ranibizumab 0.5 mg n=126	PRN ranibizumab 0.5 mg n=118
Ocular SAE, Total, n (%)	2 (1.6)	1 (0.8)	0 (0.0)
Eye disorders	1 (0.8)	0 (0.0)	0 (0.0)
Vitreous haemorrhage[†]	1 (0.8)	0 (0.0)	0 (0.0)
Infections and infestations	1 (0.8)	0 (0.0)	0 (0.0)
Endophthalmitis[¶]	1 (0.8)	0 (0.0)	0 (0.0)
Nonocular SAE, Total, n (%)	33 (26.2)	29 (23.0)	23 (19.5)
Cardiac disorders	8 (6.3)	8 (6.3)	1 (0.8)
Myocardial infarction[¥]	4 (3.2)	1 (0.8)	1 (0.8)
Acute myocardial infarction	0 (0.0)	2 (1.6)	0 (0.0)
Infections and infestations	6 (4.8)	4 (3.2)	2 (1.7)
Pneumonia	3 (2.4)	1 (0.8)	0 (0.0)
Nervous system disorders	4 (3.2)	5 (4.0)	4 (3.4)
Cerebrovascular accident	1 (0.8)	2 (1.6)	3 (2.5)
Vascular disorders	4 (3.2)	2 (1.6)	4 (3.4)
Hypertension	1 (0.8)	1 (0.8)	0 (0.0)
Deaths[*]	2 (1.6)	4 (3.2)	1 (0.8)

Safety set consisted of all patients who received at least one administration of study treatment in the study eye and had at least one postbaseline safety assessment

[†]suspected to be related to treatment and ocular injection; [¶]suspected to be related to treatment and ocular injection

[¥]Overall, myocardial infarction was reported in eight patients, of which acute myocardial infarction was reported in two patients Of the six myocardial infarction reports, two were suspected to be related to study treatment. Of the two patients with acute myocardial infarction, one was suspected to be related to study treatment

[*]Reasons for death, n (%): myocardial infarction: 2 (1.6), myocardial ischemia: 1 (0.8), lung neoplasm malignant: 1 (0.8), gastrointestinal haemorrhage: 1 (0.8), cardio-respiratory arrest: 1 (0.8), and cerebrovascular accident: 1 (0.8); For deaths related to study treatment description: in the first case, the patient had two severe occurrences of diabetic foot and the investigator did not suspect a relationship between the event and the study medication or injection procedure. The interval from the last ranibizumab treatment to myocardial infarction event leading to patient's death was 19 days. In the investigator's opinion, the other possible contributory factors leading to death included aggravation of diabetes. In the second case, the patient had a severe brain haemorrhage, stroke (cerebrovascular accident) and died on the same day. The interval from the last ranibizumab treatment to cerebrovascular accident event leading to patient's death was 12 days

A patient with multiple occurrences of a preferred term is counted only once in the preferred term row

PRN, pro re nata; SAE, serious adverse event; SD, standard deviation; T&E, treat-and-extend