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A Crossover Design for Comparative Efficacy

A 36-Week Randomized Trial of Bevacizumab and Ranibizumab for Diabetic Macular Edema

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Purpose: To investigate the comparative efficacy of bevacizumab (Avastin) and ranibizumab (Lucentis; both Genentech, Inc, South San Francisco, CA) for diabetic macular edema (DME) using a crossover study design.

Design: Randomized, double-masked, 36-week, 3-period crossover clinical trial.

Participants: Fifty-six subjects with DME involving the center of the macula in one or both eyes.

Methods: Monthly intravitreous injections of bevacizumab (1.25 mg) or ranibizumab (0.3 mg).

Main Outcome Measures: Comparison of mean changes in visual acuity and central retinal thickness, tested using a linear mixed-effects model.

Results: Based on the linear mixed-effects model, the 3-month estimated mean improvement in visual acuity was 5.3 letters for bevacizumab and 6.6 letters for ranibizumab (difference, 1.3 letters; $P = 0.039$). Estimated change in optical coherence tomography (OCT) central subfield mean thickness (CSMT) was −89 μm for bevacizumab and −137 μm for ranibizumab (difference, 48 μm; $P < 0.001$). Incorporating cumulative treatment benefit, the model yielded a predicted 36-week (9-month) average improvement in visual acuity of 7.1 letters (95% confidence interval [CI], 5.0–9.2) for bevacizumab and 8.4 letters (95% CI, 6.3–10.5) for ranibizumab, and a change in OCT CSMT of −128 μm (95% CI, −155 to −100) for bevacizumab and −176 μm (95% CI, −202 to −149) for ranibizumab. There was no significant treatment-by-period interaction (i.e., treatment difference was constant in all 3 periods), nor was there a significant differential carryover effect from one period to the next.

Conclusions: This trial demonstrated a statistically significant but small relative clinical benefit of ranibizumab compared with bevacizumab for treatment of DME, using a markedly reduced sample size relative to a full comparative efficacy study. The effects on visual acuity and central retinal thickness for the 2 drugs are consistent with those reported at 1 year for the concurrent parallel-group trial by the Diabetic Retinopathy Clinical Research Network testing bevacizumab, ranibizumab, and aflibercept for DME. The 3-period crossover design allowed for meaningful and efficient comparison, suggesting that this approach may be useful for future comparative efficacy studies of anti–vascular endothelial growth factor drugs for DME. *Ophthalmology 2016;123:841-849 © 2016 Published by Elsevier on behalf of the American Academy of Ophthalmology.*

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The comparative efficacy of bevacizumab (Avastin; Genentech, Inc, South San Francisco, CA), ranibizumab (Lucentis; Genentech), and aflibercept (Eylea, Regeneron Pharmaceuticals, Inc, Tarrytown, NY) for treatment of diabetic macular edema (DME) is being investigated in a large, randomized, parallel-group clinical trial carried out by the Diabetic Retinopathy Clinical Research Network (DRCR.net; ClinicalTrials.gov identifier, NCT01627249). Recently reported 1-year results for this study demonstrate efficacy for all 3 drugs.[1] Analysis of the primary outcome, mean change in visual acuity at 1 year, showed that there was an overall relative benefit of aflibercept compared with the other 2 drugs. However, there was a statistically significant interaction between baseline visual acuity and the treatment effect for aflibercept, warranting stratification of the results by baseline visual acuity. The treatment effect was similar among the 3 drugs for eyes with baseline visual acuity score of 69 letters or more (Snellen equivalent, approximately 20/40 or better) and demonstrated superiority of aflibercept for eyes with a baseline visual acuity score of fewer than 69 letters (Snellen equivalent, worse than 20/40).

Ranibizumab (0.3 mg) and aflibercept (2 mg) are approved by the United States Food and Drug Administration (FDA) for the treatment of DME, based on results of several randomized clinical trials.[2–5] Bevacizumab has not

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been tested for this indication in a large clinical trial before the DRCR.net study, but has been used widely off-label in recent years on the basis of benefit shown in case series and small trials,[6–11] and has shown efficacy equal to that of ranibizumab in large clinical trials for neovascular age-related macular degeneration.[12–17]

The findings of the DRCR.net trial offer invaluable and definitive guidance about the comparative efficacy of available anti–vascular endothelial growth factor (VEGF) agents for treatment of DME. Such studies are the gold standard for comparative efficacy research, but the investment necessary to execute these projects is large, and the time necessary to organize and carry out these trials is considerable.

We asked whether a crossover study design might offer a meaningful and efficient comparison of 2 intravitreally administered anti-VEGF drugs for DME, using a smaller sample size than required for a traditional parallel-group trial. We specifically wanted to compare findings from a small crossover study with those from the large comparative efficacy trial being planned by the DRCR.net. Crossover studies, in which every participant receives both treatments being compared, offer statistical efficiency that permits use of a smaller sample size than would be required for a parallel-group trial, in which each participant receives only 1 treatment being tested. Some crossover trial designs can be problematic, particularly when carryover effects (residual effects) of one drug complicate measurement of the effects of a second drug in subjects given one and then the other, making it difficult or impossible to evaluate a treatment difference. Two-period, 2-sequence designs susceptible to such problems have been criticized and are used infrequently in biomedical research.[18] However, extended crossover designs making use of additional treatment periods and sequences have been developed to overcome these shortcomings under appropriate conditions.[19,20]

The treatment effect of anti-VEGF drugs on DME, which is rapid, easily measured, and typically reversible in the short term, combined with the similarities of the drugs, seemed well suited to this design. We chose to compare bevacizumab and ranibizumab, the 2 anti-VEGF drugs most widely used for treatment of DME at the time of study initiation, and carried out this trial concurrently with the DRCR.net study to compare findings from the 2 study designs.

Methods

This randomized, double-masked, 36-week, 3-period, 2-treatment crossover clinical trial was conducted at 2 sites, the National Eye Institute, Bethesda, Maryland, and University Hospitals Bristol National Health Service Foundation Trust, Bristol, United Kingdom, with the Emmes Corporation, Rockville, Maryland, acting as the Data and Statistical Coordinating Center. Institutional review board or independent ethics committee approval was obtained at both sites, and all participants gave written informed consent. The study was conducted in accordance with the tenets of the Declaration of Helsinki. No stipend was given for participation. An independent data and safety monitoring committee provided study oversight and approved this manuscript. The study is registered at www.clinicaltrials.gov under identifier NCT01610557. This project was supported with federal funds from the National Eye Institute, National Institutes of Health, Department of Health and Human Services, under contract no. HHSN263201200001C. Patient recruitment and clinical research staff costs also were supported in the United Kingdom by the National Institute for Health Research's Clinical Research Network West of England and Moorfields Biomedical Research Center, as part of the Universities and National Institutes Transatlantic Eye consortium (UNITE).

Study Population

Eligible participants had type 1 or type 2 diabetes mellitus, were at least 18 years of age, and could enter one or both eligible eyes in the study. Principal eligibility criteria for a study eye included: (1) presence of DME involving the center of the macula, (2) Early Treatment Diabetic Retinopathy Study (ETDRS) best-corrected visual acuity letter score of 78 to 24 (Snellen equivalent, 20/32–20/400), and (3) central subfield mean thickness (CSMT) of 330 μm or more on Cirrus (Carl Zeiss Meditec, Inc, Dublin, CA) optical coherence tomography (OCT). Major exclusion criteria for the study eye included presence of factors or other conditions judged to impact the course of edema or to preclude possible improvement in vision with treatment; panretinal photocoagulation, focal or grid laser photocoagulation, or depot corticosteroid injection within the previous 3 months; ocular injection with an anti-VEGF agent within the previous 2 months; more than 4 injections with an anti-VEGF agent within the previous year; or prior vitrectomy. Potential participants were excluded for history of renal failure (requiring hemodialysis or renal transplantation) and for a measured systolic blood pressure of more than 180 mmHg or a diastolic blood pressure of more than 110 mmHg.

Study Design

This study used a randomized, double-masked, 3-period, 2-treatment crossover design with 4 treatment sequence patterns. Each of 3 12-week periods consisted of 3 intravitreous injections of ranibizumab (0.3 mg) or bevacizumab (1.25 mg), given every 4 weeks, with evaluation of the treatment period 4 weeks after the third dose (i.e., weeks 12, 24, and 36).

Each study eye received 9 monthly injections over the course of the trial, according to a pattern of treatments determined by 1 of 4 randomly assigned sequences: R-R-B, R-B-B, B-B-R, or B-R-R, where R indicates a series of 3 consecutive ranibizumab injections, and B represents a series of 3 consecutive bevacizumab injections. Participants were assigned to 1 of the 4 treatment sequences using a randomization list generated by the Data and Statistical Coordinating Center before study initiation, with balance after every 12 enrollments. The list was provided to unmasked pharmacists at each site, who confirmed a valid participant identification code before dispensing study treatment. Both clinical sites used the same randomized list, but selected treatment assignments from opposite ends. For participants entering both eyes in the trial, the right eye was assigned randomly as above to 1 of the 4 treatment sequences, and the left eye was assigned automatically to the sequence with the inverse schedule (for example, B-R-R in the right eye and R-B-B in the left eye).

Treatment

Participants and investigators were masked to treatment. Site staff collecting study data, including research coordinators, technicians, and photographers, were also masked. Bevacizumab (1.25 mg) or ranibizumab (0.3 mg) was administered every 4 weeks according to a study eye's randomly assigned schedule. Visits were scheduled

within a window of ±10 days from the target date, but treatment could not be repeated within 14 days of a previous injection. The injection protocol required use of a lid speculum and application of povidone iodine. Participants with both eyes entered in the study could elect bilateral same-day treatment or could return on a second day within the visit window for injection of the other eye.

Study eyes meeting predefined criteria for significant worsening of DME at week 12 or later could receive focal or grid laser photocoagulation. Fellow eyes in participants only enrolling 1 eye could receive any necessary ocular treatment.

Eleven doses of ranibizumab 0.5 mg were given to participants at the start of the study. After publication of 2 large trials reporting no difference in efficacy between ranibizumab 0.3 mg and 0.5 mg for DME[4] and subsequent FDA approval of the 0.3-mg dose for DME, the protocol was amended and ranibizumab 0.3 mg was used for the remainder of the study (98% of all ranibizumab injections).

Examination Procedures

Best-corrected visual acuity measured using an ETDRS chart with standardized manifest refraction was obtained at baseline (week 0) and at weeks 12, 24, and 36, corresponding to time points 4 weeks after the third injection of each 12-week period. Testing performed at all visits included visual acuity and intraocular pressure measurement, slit-lamp biomicroscopy, dilated fundus examination, and OCT scanning obtained on a Cirrus machine. Technical difficulties with a Cirrus machine mandated a protocol amendment during the study, permitting OCT scanning using a Spectralis device (Heidelberg Engineering, Inc, Heidelberg, Germany) for instances in which a Cirrus device was unavailable. The amendment stipulated collection of both Cirrus and Spectralis scans at subsequent visits at the affected site, including visits during a prespecified extension phase of the study through week 52 to allow for development and validation of a function to convert Spectralis values to Cirrus equivalents at all visits for which a Cirrus scan was not performed (please see "Data Analysis" for details). The OCT scans for all visits were graded in masked fashion at an external reading center (Duke University, Durham, NC).

Outcomes

The analysis of this crossover study tested for a difference in 12-week treatment effect between bevacizumab and ranibizumab. The primary outcome was the mean change in best-corrected visual acuity from baseline, estimated for a 3-month dosing period in a linear mixed-effects model. The main prespecified secondary outcome was the change in central retinal thickness, measured as OCT CSMT, estimated for a 3-month dosing period using the linear mixed-effects model.

Data Analysis

Differences in mean change in visual acuity and OCT CSMT were tested using a 2-sided type 3 *F* test of the treatment effect in a linear mixed-effects regression model, where the final model included fixed-effects for treatment (bevacizumab or ranibizumab), period (1, 2, or 3), clinical site (National Eye Institute or Bristol), and baseline visual acuity score; and with random effects for subject and eye nested within subject.[19] The model was fit using the GLIMMIX procedure using SAS software (SAS Inc, Cary, NC). Protocol-defined model building steps included evaluation of first-order carryover effect (i.e., effect of treatment received in the preceding period, where applicable), period-by-treatment interactions, sequence effects, and sequence-by-period interactions, none of which were found to be significant or to have substantive impact on the estimated effect of treatment when included in the model. Twelve- and 36-week changes in visual acuity and OCT CSMT are model estimates based on data from all subjects and eyes and all treatment periods. This 4-sequence design has been shown to provide unbiased estimates of treatment and first-order carryover effects (where carryover effects that persist for only 1 period are termed *first-order effects*, those for 2 periods are termed *second-order effects*, and so on) and is considered to be the optimal 3-period, 2-treatment, 4-sequence design for estimating treatment differences in the presence of differential or symmetric first-order carryover effects.[20,21]

Stratified analysis of eyes with baseline ETDRS visual acuity letter score of 69 letters or more (Snellen equivalent, approximately 20/40 or better) and eyes with baseline score of less than 69 letters (Snellen equivalent, worse than 20/40) was not prespecified in the analytic plan. The DRCR.net trial published 1-year results using such stratification, on the basis of a significant interaction between baseline visual acuity and treatment effect for aflibercept, so we added a similar analysis to allow for additional comparison with the DRCR.net results.

In approximately 12% of key visits (i.e., week 12, 24, or 36), participants underwent Spectralis OCT scanning rather than the prespecified Cirrus OCT testing because of technical difficulties with a Cirrus device. After repair of the Cirrus device, both Cirrus and Spectralis OCT scans were captured at 150 subsequent participant visits, enabling development and validation of a linear conversion function for CSMT from Spectralis to Cirrus devices, similar to work performed previously by the DRCR.net.[22] Prediction error of the conversion function was evaluated using a bootstrap cross-validation routine and was estimated to be 8.4 μm (95% confidence interval [CI], 8.4−8.6 μm). Spectralis values were converted and used as Cirrus CSMT values for the 12% of key visits at which the Cirrus scan was not performed. A worst-case sensitivity analysis, adding and subtracting twice the prediction error for imputed CSMT values for observations in the ranibizumab and bevacizumab groups, respectively, did not impact the statistical significance and reduced the estimated effect size by less than 5.6% (i.e., 2.7 μm).

The study sample size was determined through simulation and used the exact model and outcomes as described above, but assumed only a single eye per participant. Within- and between-subject standard deviations were each assumed to be 5 ETDRS letters (0.1 logMAR). A differential first-order carryover effect of 20% (i.e., 20% of the effect of the previous period would be maintained through a subsequent period) was assumed. Under these conditions, a study of 60 eyes was expected to have 87% power to detect a 2.5-letter (0.05-logMAR) difference between treatments, rising to 89% if no carryover effect was present.

Results

Fifty-six participants were enrolled in the study between June 2012 and January 2014, including 6 participants with both eyes enrolled. One participant with a single eye assigned to the R-B-B group withdrew after the week 4 visit after a hemorrhagic stroke. All remaining participants completed the study, including the week 12, 24, and 36 visits, and were included in this analysis.

Baseline characteristics for all participants are shown in Table 1. The largest imbalances among the 4 study groups were for participants or eyes assigned to the R-B-B sequence. Compared with the overall mean, age was 2.9 years older, hemoglobin A1c was 0.2% higher, visual acuity was 3 letters lower, and OCT CSMT was 33 μm less in this group.

All participants received study medication according to their randomly assigned schedule, 92% (449/487 injections) given within the protocol-specified window of ±10 days. No study eye demonstrated significant worsening of DME or received

Table 1. Baseline Characteristics

Variable and Category	RRB	RBB	BBR	BRR	Total
Total no. of eyes	17	15*	16	14	62
Age (yrs)					
Minimum	39	39	39	51	39
Median / **Mean**	61 / **62.4**	66 / **65.9**	63 / **62.3**	61.5 / **61.8**	62 / **63**
Maximum	85	87	83	82	87
Diabetes type, no. (%)					
1	2 (11.8)	2 (13.3)	1 (6.3)	2 (14.3)	7 (11.3)
2	15 (88.2)	13 (86.7)	15 (93.8)	12 (85.7)	55 (88.7)
Hemoglobin A1c (%)					
Minimum	6.2	5.8	6.2	5.8	5.8
Median / **Mean**	7.4 / **8.1**	7.8 / **8.4**	7.9 / **7.9**	7.6 / **7.8**	7.6 / **8.1**
Maximum	11.5	12.2	10.6	10.3	12.2
≥8.0%, no. (%)	6 (35)	6 (40)	7 (43)	5 (35)	24 (38)
Gender, no. (%)					
Female	4 (23.5)	8 (53.3)	7 (43.8)	5 (35.7)	24 (38.7)
Male	13 (76.5)	7 (46.7)	9 (56.3)	9 (64.3)	38 (61.3)
Race, no. (%)					
Asian	1 (5.9)	0 (0)	1 (6.3)	1 (7.1)	3 (4.8)
Black (participant reported)	2 (11.8)	2 (13.3)	2 (12.5)	2 (14.3)	8 (12.9)
Multiple race	1 (5.9)	0 (0)	2 (12.5)	0 (0)	3 (4.8)
Unknown	1 (5.9)	0 (0)	0 (0)	1 (7.1)	2 (3.2)
White	12 (70.6)	13 (86.7)	11 (68.8)	10 (71.4)	46 (74.2)
Ethnicity					
Unknown	0 (0)	0 (0)	1 (6.3)	0 (0)	1 (1.6)
Hispanic or Latino (participant reported)	2 (11.8)	0 (0)	1 (6.3)	0 (0)	3 (4.8)
Not Hispanic or Latino	15 (88.2)	15 (100)	14 (87.5)	14 (100)	58 (93.5)
Best-corrected visual acuity (letters)					
Minimum	38	32	44	50	32
Median / **Mean**	69 / **65**	64 / **61**	69 / **65**	64 / **65**	66 / **64**
Maximum	78	73	75	78	78
Central subfield mean thickness measured by OCT (μm)					
Minimum	334	366	362	358	334
Median / **Mean**	496 / **484**	435 / **444**	432 / **471**	508 / **508**	453 / **477**
Maximum	720	602	606	653	720

B = bevacizumab; R = ranibizumab.
Participants with both eyes enrolled are counted twice (by eye), once for each of the treatment sequences to which an eye was assigned randomly.
*One participant with a single eye assigned to the RBB group withdrew after the week 4 visit after a hemorrhagic stroke and was not included in the analysis; all other participants and eyes completed the study and were included in the analysis.

supplemental application of focal or grid laser photocoagulation or other adjuvant treatment for DME.

Based on the linear mixed-effects model, the 3-month estimated mean improvement in visual acuity was 5.3 letters (95% CI, 3.2−7.4 letters) for bevacizumab and 6.6 letters (95% CI, 4.5−8.7 letters) for ranibizumab, with an estimated difference of 1.3 letters (95% CI, 0.07−2.5 letters; $P = 0.039$) (Table 2). Model-based estimates of mean change in CSMT measured by OCT were −89 μm (95% CI, −116 to −62 μm) for bevacizumab and −137 μm (95% CI, −164 to −110 μm) for ranibizumab, with an estimated difference of −48 μm (95% CI, −65 to −31 μm; $P < 0.001$) (Table 2). Figure 1 presents changes in visual acuity (Fig 1A) and OCT CSMT (Fig 1B) from baseline for periods 1, 2, and 3 by treatment group, illustrated as individual measurements, raw means, and model-based estimates from the mixed-effects analysis for each drug.

A significant period effect was identified, indicating a cumulative benefit over time with either drug. For every 3-month period, improvement in visual acuity attributable to the period effect was estimated to be 0.9 letters (95% CI, 0.2−1.6 letters) and decrease in OCT CSMT attributable to the period effect was estimated to be 19 μm (95% CI, 9−29 μm), whether receiving bevacizumab or ranibizumab.

Combining the period and treatment effects in the mixed-effects model yielded a predicted 9-month (36-week) average improvement in visual acuity of 7.1 letters (95% CI, 5.0−9.2 letters) for bevacizumab and 8.4 letters (95% CI, 6.3−10.5 letters) for ranibizumab, and a predicted 9-month average decrease in OCT CSMT of 128 μm (95% CI, 100−155 μm) for bevacizumab and 176 μm (95% CI, 149−202 μm) for ranibizumab.

There was no significant treatment-by-period interaction, implying that differences between ranibizumab and bevacizumab were similar in all periods. There was no significant differential first-order carryover effect at outcome measurement at weeks 12, 24, and 36. Figure 2 shows the estimated differential first-order carryover effect on change in visual acuity (Fig 2A) and OCT CSMT (Fig 2B) at 4, 8, and 12 weeks after crossover, illustrated as a difference between the effect of ranibizumab and bevacizumab from the previous period, with the corresponding treatment effect for the present period shown for comparison. At 4 weeks, this figure shows that the treatment received in the preceding period had a greater impact on outcome than the

Table 2. Results of Crossover (Mixed-Effects Model) Analysis

	Bevacizumab	Ranibizumab	Difference	*P* Value
Best-corrected visual acuity, **change from baseline** (letters)	5.3 (3.2−7.4)	6.6 (4.5−8.7)	1.3 (0.07−2.5)	0.039
Best-corrected visual acuity (letters)	69.5 (67.4−71.6)	70.8 (68.7−72.9)		
Central subfield mean thickness assessed by OCT, **change from baseline** (μm)	−89 (−116 to −62)	−137 (−164 to −110)	−48 (−65 to −31)	<0.001
Central subfield mean thickness assessed by OCT (μm)	388 (361−415)	340 (313−367)		

OCT = optical coherence tomography.
Data are mean (95% confidence interval). Bevacizumab and ranibizumab columns represent the estimated effect of the drug for a 3-month period, adjusted for period and baseline value. The difference column represents the estimated difference between the 2 drugs.

treatment received in the current period. This was reversed by 8 weeks and further decreased by 12 weeks, when the treatment received in the current period dominated the nonsignificant impact of treatment received in the preceding period.

We evaluated how many eyes achieved a normal or near-normal CSMT within the first 24 weeks and maintained this level of improvement through 36 weeks. Six of 31 eyes (19%) receiving ranibizumab and 2 of 30 eyes (7%) receiving bevacizumab achieved an OCT CSMT of less than 275 μm in the first period (12 weeks) and maintained a CSMT of less than 275 μm through 36 weeks. Considering eyes that did not achieve a CSMT of less than 275 μm during the first period, but did so in the second period and maintained this improvement through 36 weeks, there were 2 of 15 eyes (13%) assigned to bevacizumab in both periods (B-B); 2 of 13 eyes (15%) assigned to bevacizumab in the first period and ranibizumab in the second period (B-R); 2 of 14 eyes (14%) assigned to ranibizumab in both periods (R-R); and 0 of 11 eyes (0%) assigned to ranibizumab in the first period and bevacizumab in the second period (R-B). Through week 12, the group rate was higher among those receiving ranibizumab, but this difference could have been achieved by chance ($P = 0.25$, Fisher exact test). Through week 24, the rate for those receiving bevacizumab for 2 consecutive periods was similar to that of those receiving ranibizumab.

An exploratory analysis, performed to allow additional comparison with 1-year results of the DRCR.net study, showed a statistically significant interaction between baseline visual acuity and the difference in treatment effect for bevacizumab and ranibizumab. Baseline characteristics for all participants, stratified by baseline visual acuity for all study eyes, are shown in Table S1 (available at www.aaojournal.org). The linear mixed-effects model was used for each of 2 strata to estimate 3-month mean improvement in visual acuity and mean change in OCT CSMT for bevacizumab and ranibizumab, with analysis stratified for eyes with baseline visual acuity score of 69 letters or more (Snellen equivalent, 20/40 or better) and for eyes with visual acuity score of less than 69 letters (Snellen equivalent, worse than 20/40). Three-month estimates, stratified by baseline visual acuity, are shown in Table 3 and include the nonstratified values for all eyes for comparison. As in the primary analysis, combination of treatment and period effects allowed estimation of 36-week (9-month) changes in visual acuity and retinal thickness. For better-seeing eyes, the average improvement in visual acuity was 4.9 letters

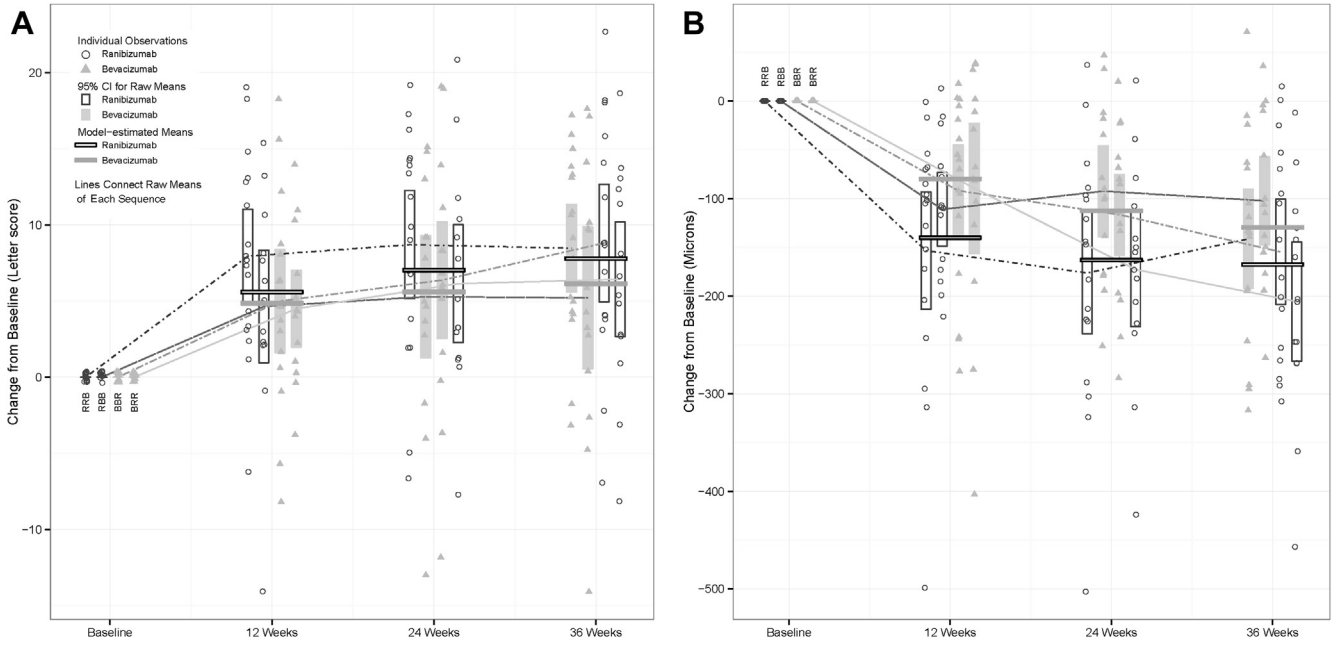

Figure 1. Graphs showing changes in (**A**) visual acuity and (**B**) central subfield mean thickness assessed by optical coherence tomography from baseline for crossover periods 1, 2, and 3 by treatment group. CI = confidence interval.

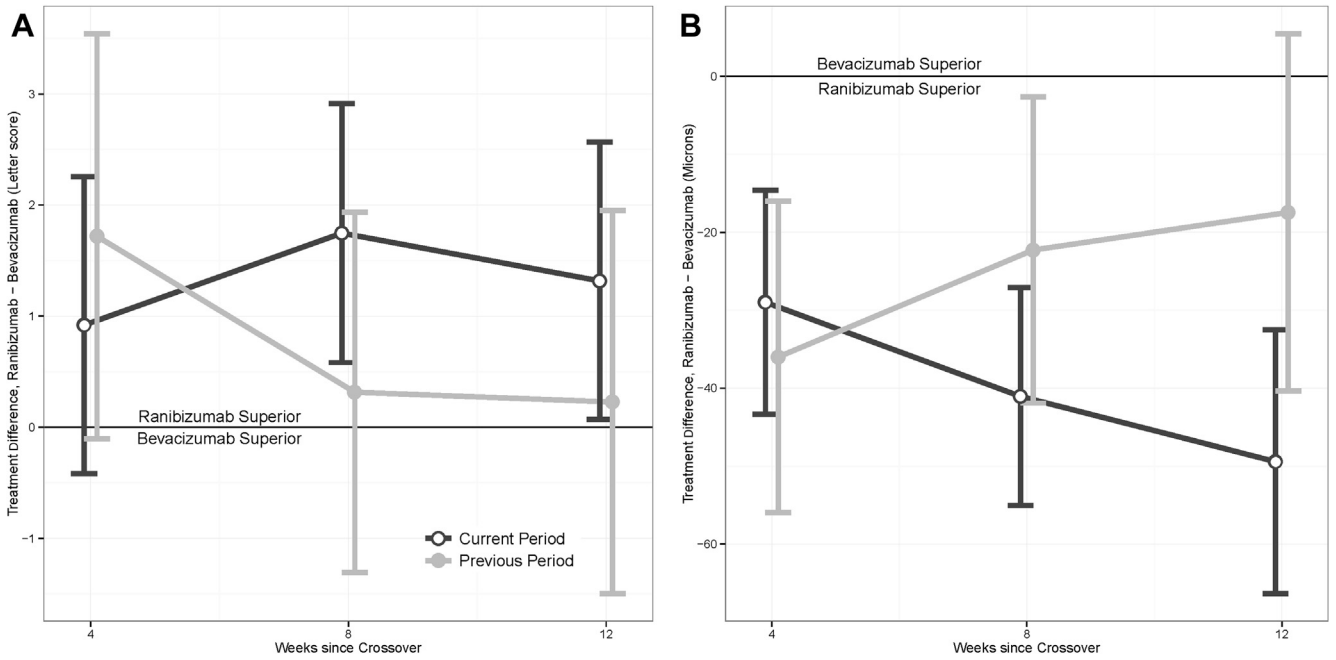

Figure 2. Graphs showing the differential first-order carryover effect (residual effect of the drug from the preceding period), shown as a treatment effect difference between ranibizumab and bevacizumab given in the previous period for (**A**) change in visual acuity and (**B**) central subfield mean thickness assessed by optical coherence tomography at 4, 8, and 12 weeks after crossover. The treatment difference attributable to the first-order carryover effect is shown in gray. The treatment difference attributable to drugs in the current period (i.e., the differential effect of the 2 drugs as estimated in the primary analysis of the study) is shown in black for comparison. Note that the treatment difference between ranibizumab and bevacizumab for the current period (in black) at 12 weeks after crossover (at outcome assessment) is the result estimated for 3 and 9 months in the primary analysis (1.3 letters [$P = 0.039$] and −48 μm [$P < 0.001$]), both favoring ranibizumab).

(95% CI, 2.0−7.8 letters) for bevacizumab and 5.3 letters (95% CI, 2.4−8.2 letters) for ranibizumab, and the change in OCT CSMT was −144 μm (95% CI, −182 to −106 μm) for bevacizumab and −184 μm (95% CI, −221 to −147 μm) for ranibizumab at 36 weeks. For worse-seeing eyes, average improvement in visual acuity was 8.6 letters (95% CI, 6.0−11.2 letters) for bevacizumab and 10.5 letters (95% CI, 7.9−13.1 letters) for ranibizumab, and the change in OCT CSMT was −117 μm (95% CI, −150 to −84 μm) for bevacizumab and −170 μm (95% CI, −203 to −137 μm) for ranibizumab at 36 weeks. Note that, as for the primary analysis, 9-month differences between the 2 drugs are equivalent to the 3-month differences shown in Table 3.

Adverse Events

There were no cases of endophthalmitis, retinal detachment, traumatic cataract, or vision loss of 15 letters or more. A single instance of hemorrhagic stroke occurred in a participant who received ranibizumab at baseline (week 0) and week 4, 18 days after the second injection.

Discussion

This randomized crossover clinical trial demonstrated a statistically significant, but small (1.3-letter difference in visual acuity, 48-μm difference in CSMT), relative estimated benefit of ranibizumab compared with bevacizumab for treatment of DME. By comparison, the large, ongoing parallel-group trial performed by the DRCR.net found a 1-year benefit of ranibizumab of 1.4 letters ($P = 0.12$, not statistically significant) and 51 μm ($P < 0.001$, statistically significant) relative to bevacizumab,[1] results essentially identical to those obtained in our study. Although caution is warranted in comparing results of an estimated 3-month period in our study with the 12-month findings in the DRCR.net trial, our analysis allows estimation of the treatment difference at 36 weeks (9 months). The rapid development of a large treatment benefit during the first several months of serial injections, with maintenance of the effect thereafter, is characteristic of the available ophthalmic anti-VEGF drugs across a number of indications,[1−5,10,12−17,23] making 9- and 12-month results very similar in these studies, including the present DRCR.net trial.

Compared with parallel-group trials, crossover trials achieve similar statistical power with fewer participants by using each subject as his or her own control. The increase in power comes at the cost of additional assumptions that are not necessary in a randomized parallel-group trial. The principal and primary assumption of all crossover studies is that the condition to be treated, whether stable or progressive over the course of the trial, would revert to the untreated state (or close approximation) if an effective intervention were ceased. That is, it is assumed that the interventions tested are not curative during the period of treatment. This assumption seems justified based on clinical experience with treatment of DME with anti-VEGF agents during the first year of treatment and is corroborated by the

Table 3. Results of Crossover (Mixed-Effects Model) Analysis, Stratified by Visual Acuity at Baseline (Post Hoc Analysis)

	Baseline Visual Acuity Score (Letters)	Bevacizumab	Ranibizumab	Difference	*P* Value
Best-corrected visual acuity, change from baseline (letters)	Any (all eyes)	5.3 (3.2–7.4)	6.6 (4.5–8.7)	1.3 (0.07–2.5)	0.039
	≥69	**3.1 (0.2–6.0)**	**3.6 (0.7–6.5)**	**0.45 (−1.4 to 2.3)**	**0.64**
	<69	**6.9 (4.3–9.4)**	**8.7 (6.2–11.3)**	**1.9 (0.3–3.5)**	**0.022**
Central subfield mean thickness assessed by OCT, **change from baseline** (μm)	Any (all eyes)	−89 (−116 to −62)	−137 (−164 to −110)	−48 (−65 to −31)	<0.001
	≥69	**−106 (−144 to −68)**	**−145 (−183 to −108)**	**−40 (−66 to −14)**	**0.0032**
	<69	**−78 (−111 to −45)**	**−132 (−165 to −99)**	**−54 (−76 to −31)**	**<0.001**

Data are mean (95% confidence interval). Bevacizumab and ranibizumab columns represent the estimated effect of the drug for a 3-month period, adjusted for period and baseline value. The difference column represents the estimated difference between the 2 drugs. Bold values denote data that was not presented in Table 2 in the nonstratified analysis. Nonbold values are presented in Table 2, but are re-iterated here for ease of comparison.

results of the DRCR.net trial, in which study eyes receiving bevacizumab or ranibizumab required a median of 10 injections (of a possible 13) in the first year, in the context of a complex re-treatment algorithm.[1] We evaluated the rate of potential cure in our study by considering eyes that achieved an OCT CSMT of less than 275 μm during the first or second period of treatment and maintained this resolution of central edema through 36 weeks. Even conservatively defining all such eyes as cured (and not simply dependent on continued monthly treatment to maintain improvement), we found that the rate of such cure is low and occurs slightly more often with ranibizumab. If this difference is real, the analysis is biased toward a reduced effect of ranibizumab, implying that the result presented here is a conservative estimate of the superiority of ranibizumab. However, the statistical significance of our results and their similarity to those of the DRCR.net trial suggest that our analysis was not meaningfully compromised or influenced by cure of eyes in the study.

There are many different crossover study designs, and each relies on different assumptions about carryover effects. A differential carryover effect (or residual effect) occurs when an intervention from a preceding period influences the assessment of treatment differences in the current period. Carryover effects that persist for only 1 period are termed *first-order effects*, those that persist for 2 periods are termed *second-order effects*, and so on. All 2-treatment, 2-period crossover designs (e.g., AB/BA or AA/AB/BB/BA designs) assume no carryover effect; violation of this assumption is a common critique of many such studies.[24] Extended, or higher-order, crossover designs can provide unbiased estimates of treatment effects when first-order carryover effects are present. For example, a 2-treatment, 3-period, 3-sequence AAB/BBA design is optimal in the presence of a first-order carryover effect, but is invalid in the presence of a second-order carryover effect or a treatment-by-period interaction.[20] The 2-treatment, 3-period, 4-sequence AAB/ABB/BBA/BAA design used in this study has been shown to be unbiased and near optimal in the presence of a simple first-order carryover effect and robust even when small second-order carryover effects or more complex treatment-by-period interactions exist.[25]

In crossover studies, a washout interval between periods is included to mitigate the possibility of carryover effects and to reduce the possibility that treatments from prior periods influence outcome measures of the current period. In a typical washout phase, participants receive no doses of investigational product for a certain interval, often designated as 5 times the half-life of the drug.[26,27] Both bevacizumab and ranibizumab have an intraocular half-life of less than 10 days.[28,29] In DME, with effective therapy available and the possibility of permanent damage to vision without treatment, a pure washout interval has the potential to compromise the care of participants. This study used an active washout, in which patients were treated every month, but the intervals between primary outcome assessments were 12 weeks (84 days) apart. Further, because the last dose in each series of 3 injections was 4 weeks before each outcome assessment, each outcome assessment occurred 16 weeks (112 days) after the last dose of the previous period. Although clinical benefit likely exceeds bioavailability of either drug in the vitreous, Figure 2 shows the magnitude and diminution of the carryover effect and demonstrates that the 12-week active washout period effectively limits the impact of treatments in prior periods on outcome assessment of the current period.

Although rare in ophthalmology, crossover trials are common in other areas of medical research.[30–36] Regulatory guidance documents for clinical evaluation of drugs for proarrhythmic potential and, more generally, for support of a New Drug Application to the FDA fully integrate crossover studies as valid for evaluation of drug effects and treatment differences under appropriate circumstances.[26,37] Specifically, the FDA Guideline for the Format and Content of the Clinical and Statistical Sections of an Application recommends consideration of "the likelihood of spontaneous change in disease during the study, and need (or lack of need) for re-establishment of baseline between treatment periods, or a plan to estimate residual effects to show that they are inconsequential" when evaluating suitability of a crossover study design.[37] The International Conference on Harmonisation Statistical Principles for Clinical Trials discusses appropriate use of crossover designs, indicating that "the disease under study be chronic and stable" and that the problem of unequal carryover will bias direct treatment comparisons in a 2-period, 2-sequence design, but that this problem is "less acute in higher order designs."[38] These considerations were critical in designing and analyzing this study. In particular, the first-order differential

carryover (residual) effect was shown to be inconsequential, and the carryover effect common to both drugs did not bias estimates of treatment effect difference.

The route for approval of novel anti-VEGF agents and of existing drugs evaluated for new indications typically involves comparison with a single, already-approved medication, not comparison with off-label drugs like bevacizumab (which is a drug of interest because of cost considerations), and not comparison with multiple agents in the class. Parallel-group trials remain the gold standard for comparison of these drugs, but the expense of these trials limits comparative efficacy research. In the setting of an expanding number of anti-VEGF drugs for an increasing array of ophthalmic indications, it may not be practical to execute a large traditional trial to provide guidance in every instance, particularly for less common diseases. Given the similarity between our findings and those of the large DRCR.net trial, we believe that the crossover design used in this study merits further evaluation as a potentially rapid and economic means of obtaining data on the comparative efficacy of anti-VEGF drugs for diseases amenable to such analysis. The appropriate circumstances for a crossover study need to be assessed carefully. If a course of anti-VEGF therapy for a given condition results in a high frequency of disease-modifying effects shortly after treatment initiation, a crossover approach is not appropriate. For example, choroidal neovascularization secondary to myopic degeneration frequently exhibits a durable response to treatment with anti-VEGF drugs within a few injections (with resolution of exudation visualized by OCT and angiography that often does not recur after cessation of treatment),[39,40] precluding use of a crossover design in this setting. In addition to consideration of factors related to the disease in question and the nature of the treatment effects to be compared, it is important to bear in mind that this design has very low statistical power to assess adverse experiences. A study of this kind seems most appropriate when there is experience with the drugs from previous large trials.

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Abbreviations and Acronyms:
CI = confidence interval; **CSMT** = central subfield mean thickness; **DME** = diabetic macular edema; **DRCR.net** = Diabetic Retinopathy Clinical Research Network; **ETDRS** = Early Treatment Diabetic Retinopathy Study; **FDA** = Food and Drug Administration; **OCT** = optical coherence tomography; **VEGF** = vascular endothelial growth factor.

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