

Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Rosenfeld PJ, Brown DM, Heier JS, et al. Ranibizumab for neovascular age-related macular degeneration. *N Engl J Med* 2006;355:1419-31.

Table 1. Eligibility Criteria for MARINA Study

Inclusion Criteria

- Age 50 years or older.
- Active primary or recurrent subfoveal lesions with choroidal neovascularization (CNV) secondary to age-related macular degeneration (AMD) in the study eye. “Active” was defined as meeting *any* of the following criteria: (1) exhibiting a $\geq 10\%$ increase in lesion size, as determined by comparing a fluorescein angiogram performed within 1 month preceding Day 0, inclusive, with a fluorescein angiogram performed within 6 months preceding Day 0, inclusive; or (2) resulting in a visual acuity loss of >1 Snellen line (or equivalent) and occurring at any time within the prior 6 months; or (3) subretinal hemorrhage associated with CNV within 1 month preceding Day 0.
- Lesions with occult CNV component are permissible. However, if classic CNV (well-demarcated hyperfluorescence boundaries in the early phase of the fluorescein angiogram) is present, the area of classic CNV must be less than 50% of the total lesion size.
- The total area of CNV (including both classic and occult components) encompassed within the lesion must be 50% or more of the total lesion area.
- The total lesion area must be 12 disc areas or less in size.
- Best corrected visual acuity, using Early Treatment of Diabetic Retinopathy Study (ETDRS) charts, of 20/40 to 20/320 (Snellen equivalent) in the study eye.

Exclusion Criteria

- Prior treatment with verteporfin photodynamic therapy, external-beam radiation therapy, or transpupillary thermotherapy in the study eye.
 - Treatment with verteporfin photodynamic therapy in the nonstudy eye less than 7 days preceding day 0.
 - Previous participation in a clinical trial (for either eye) involving antiangiogenic drugs (pegaptanib, ranibizumab, anecortave acetate, protein kinase C inhibitors, etc.)
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Table 1. Eligibility Criteria for MARINA Study (cont'd)

Exclusion Criteria (cont'd)

- Previous intravitreal drug delivery (e.g., intravitreal corticosteroid injection or device implantation) in the study eye.
 - Previous subfoveal focal laser photocoagulation in the study eye.
 - Laser photocoagulation (juxtafoveal or extrafoveal) in the study eye within 1 month preceding day 0.
 - History of vitrectomy surgery in the study eye.
 - History of submacular surgery or other surgical intervention for AMD in the study eye.
 - Previous participation in any studies of investigational drugs within 1 month preceding day 0 (excluding vitamins and minerals).
 - Subretinal hemorrhage in the study eye that involves the fovea, if the size of the hemorrhage is either 50% or more of the total lesion area or 1 or more disc areas in size.
 - Subfoveal fibrosis or atrophy in the study eye.
 - CNV in either eye due to other causes, such as ocular histoplasmosis, trauma, or pathologic myopia.
 - Retinal pigment epithelial tear involving the macula in the study eye.
 - Any concurrent intraocular condition in the study eye (e.g., cataract or diabetic retinopathy) that, in the opinion of the investigator, could either (a) require medical or surgical intervention during the 24-month study period to prevent or treat visual loss that might result from that condition, or (b) if allowed to progress untreated, could likely contribute to loss of at least 2 Snellen equivalent lines of best corrected visual acuity over the 24-month study period.
 - Active intraocular inflammation (grade trace or above) in the study eye.
 - Current vitreous hemorrhage in the study eye.
 - History of rhegmatogenous retinal detachment or macular hole (Stage 3 or 4) in the study eye.
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Table 1. Eligibility Criteria for MARINA Study (cont'd)

Exclusion Criteria (cont'd)

- History of idiopathic or autoimmune-associated uveitis in either eye.
 - Infectious conjunctivitis, keratitis, scleritis, or endophthalmitis in either eye.
 - Aphakia or absence of the posterior capsule in the study eye.
 - Spherical equivalent of the refractive error in the study eye demonstrating more than –8 diopters of myopia.
 - Intraocular surgery (including cataract surgery) in the study eye within 2 months preceding day 0.
 - Uncontrolled glaucoma in the study eye (defined as intraocular pressure of 30 mmHg or more despite treatment with antiglaucoma medications).
 - History of glaucoma filtering surgery in the study eye.
 - History of corneal transplant in the study eye.
 - Premenopausal women not using adequate contraception.
 - History of other disease, metabolic dysfunction, physical examination finding, or clinical laboratory finding giving reasonable suspicion of a disease or condition that contraindicates the use of an investigational drug or that might affect interpretation of the results of the study or render the subject at high risk for treatment complications.
 - Current treatment for active systemic infection.
 - History of allergy to fluorescein, not amenable to treatment with diphenhydramine.
 - Inability to obtain fundus photographs or fluorescein angiogram of sufficient quality to be analyzed and graded by the central reading center.
 - Inability to comply with study or follow-up procedures.
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Table 2. Patient Disposition in MARINA Trial

	Sham	Ranibizumab 0.3 mg	Ranibizumab 0.5 mg	Total
Enrolled	—	—	—	716 (100)
Randomly assigned to treatment	238 (100)	238 (100)	240 (100)	716 (100)
Received randomized treatment*	236 (99.2)	238 (100)	239 (99.6)	713 (99.6)
Intent-to-treat patients for efficacy analyses	238 (100)	238 (100)	240 (100)	716 (100)
Included in safety evaluation	236 (99.2)	238 (100)	239 (99.6)	713 (99.6)
Completed Month 12 [†]	212 (89.1)	226 (95.0)	226 (94.2)	664 (92.7)
Crossed over from Sham to 0.5 mg ranibizumab	12 (5.0)	—	—	—
At Month 22	5 (2.1)	—	—	—
At Month 23	7 (2.9)	—	—	—
Completed Study	190 (79.8)	210 (88.2)	215 (89.6)	615 (85.9)
Discontinued from study	48 (20.2)	28 (11.8)	25 (10.4)	101 (14.1)
Death	5 (2.1)	5 (2.1)	6 (2.5) [‡]	16 (2.2)
Adverse event	8 (3.4) [§]	3 (1.3)	5 (2.1)	16 (2.2)
Lost to follow-up	2 (0.8)	3 (1.3)	3 (1.3)	8 (1.1)
Patient's decision	20 (8.4)	15 (6.3)	10 (4.2)	45 (6.3)
Physician's decision	1 (0.4)	0	1 (0.4)	2 (0.3)
Noncompliance	1 (0.4)	1 (0.4)	0	2 (0.3)
Patient's condition mandated other therapeutic intervention [¶]	11 (4.6)	1 (0.4)	0	12 (1.7)

Table 2. Patient Disposition in MARINA Trial (cont'd)

	Sham	Ranibizumab 0.3 mg	Ranibizumab 0.5 mg	Total
Discontinued treatment [#]	68 (28.6)	30 (12.6)	33 (13.8)	131 (18.3)
Death	5 (2.1)	5 (2.1)	3 (1.3) [‡]	13 (1.8)
Adverse event	13 (5.5)	8 (3.4)	15 (6.3) [‡]	36 (5.0)
Lost to follow-up	2 (0.8)	2 (0.8)	3 (1.3)	7 (1.0)
Patient's decision	25 (10.5)	17 (7.1)	13 (5.4)	55 (7.7)
Physician's decision	2 (0.8)	1 (0.4)	2 (0.8)	5 (0.7)
Noncompliance	1 (0.4)	0	0	1 (0.1)
Patient's condition mandated other therapeutic intervention ^{††}	23 (9.7)	1 (0.4)	0	24 (3.4)

*Reasons for not receiving randomized treatment included patient became apprehensive about intravitreal injections (1 Sham), and patient was randomized ahead of the schedule before the safety eligibility confirmation for an intravitreal injection (1 each for Sham and Ranibizumab 0.5 mg).

† Defined as having the visual acuity assessment at Month 12. Data from patients who missed the Month 12 visit but stayed in the study for the second year were not counted. The visual acuity score at Month 12 for one patient in the 0.5-mg ranibizumab group was obtained at 4 meters only.

‡ Three patients discontinued from treatment because of an adverse event that resulted in death (the primary reason for study discontinuation).

§ One patient discontinued from study because of congestive heart failure and died on the same date of the event.

†† The mandated therapeutic interventions included verteporfin photodynamic therapy with or without triamcinolone acetonide injection and intravitreal injection of pegaptanib sodium.

Some patients remained in the study after treatment discontinuation.

Table 3. Criteria for Serious (Sight-Threatening) Ocular Adverse Events*

- Adverse event causes a decrease in visual acuity of ≥ 30 letters (compared with the last assessment of visual acuity prior to the most recent treatment) > 1 hour.
- Adverse event causes a decrease in visual acuity to the level of Light Perception or worse lasting > 1 hour *post-injection/sham procedure*.
- Adverse event requires surgical intervention (e.g., conventional surgery, vitreous tap or biopsy with intravitreal injection of anti-infectives, or laser or retinal cryopexy with gas) to prevent permanent loss of sight.
- Adverse event is associated with severe intraocular inflammation (i.e., 4+ anterior chamber cell/flare or 4+ vitritis).
- In the opinion of the investigator, adverse event may require medical intervention to prevent permanent loss of sight.

*An adverse event meeting any one of these five criteria was considered serious.

Table 4. Grading Scales for Flare/Cells*

Flare	
0	No protein is visible in the anterior chamber when viewed by an experienced observer using slit lamp biomicroscopy; a small, bright, focal slit-beam of white light; and high magnification.
Trace	Trace amount of protein detectable in the anterior chamber. This protein is visible only with careful scrutiny by an experienced observer using slit lamp biomicroscopy; a small, bright, focal slit-beam of white light; and high magnification.
1+	Mild amount of protein detectable in the anterior chamber. The presence of protein in the anterior chamber is immediately apparent to an experienced observer using slit lamp biomicroscopy and high magnification, but such protein is detected only with careful observation with the naked eye and a small, bright, focal slit-beam of white light.
2–3+	Moderate amount of protein detectable in the anterior chamber. These grades are similar to 1+ but the opacity would be readily visible to the naked eye of an observer using any source of a focused beam of white light. This is a continuum of moderate opacification, with 2+ being less apparent than 3+.
4+	A large (severe) amount of protein is detectable in the anterior chamber. Similar to 3+, but the density of the protein approaches that of the lens. Additionally, frank fibrin deposition is frequently seen in acute circumstances. It needs to be noted that because fibrin may persist for a period of time after partial or complete restoration of the blood–aqueous barrier, it is possible to have resorbing fibrin present with lower numeric assignments for flare (e.g., 1+ flare with fibrin).

Table 4. Grading Scales for Flare/Cells* (cont'd)

Cells	
0	No cells are seen in any optical section when a large slit lamp beam is swept across the anterior chamber.
Trace	Rare (1–3) cells are observed when the slit lamp beam is swept across the anterior chamber. When the instrument is held stationary, not every optical section contains circulating cells.
1+	3–10 cells/optical section are seen when the slit-beam of light sweeps across the anterior chamber. When the instrument is held stationary, every optical section contains circulating cells.
2+	10–25 cells are seen when the slit-beam of light sweeps across the anterior chamber. When the instrument is held stationary, every optical section contains circulating cells.
3+	25–50 cells are seen when the slit-beam of light sweeps across the anterior chamber. When the instrument is held stationary, every optical section contains circulating cells. Keratic precipitates or cellular deposits on the anterior lens capsule may be present.
4+	More than 50 cells are seen when the slit-beam of light sweeps across the anterior chamber. When the instrument is held stationary, every optical section contains cells, or hypopyon is noted. As for fibrin deposition, hypopyon may persist for some period of time after the active exudation of cells into the anterior chamber has diminished or ceased entirely, making it possible to have 1+ circulating cells in the anterior chamber with a resolving hypopyon.

*Modified from Hogan MH, Kimura SJ, Thygeson P. Signs and symptoms of uveitis. I. Anterior uveitis. Am J Ophthalmol 1959;47:155.²²

Table 5. Grading Scale for Vitreous Cells*†

Cells in Retroilluminated Field	Description	Grade
0–1	Clear	0
2–20	Few opacities	Trace
21–50	Scattered opacities	1
51–100	Moderate opacities	2
101–250	Many opacities	3
>251	Dense opacities	4

*Using a Hruby lens.

†Nussenblatt RB, Whitcup SM, Palestine AG. Uveitis: fundamentals and clinical practice. 2nd rev. ed. New York: Mosby, 1996, p. 64.²³

Table 6. Nonocular Hemorrhagic Adverse Events

MedDRA* Preferred Term	Sham (n = 236)	Ranibizumab 0.3 mg (n = 238)	Ranibizumab 0.5 mg (n = 239)
Total† — no. (%)	13 (5.5)	22 (9.2)	21 (8.8)
Epistaxis	3 (1.3)	5 (2.1)	4 (1.7)
Hematoma	3 (1.3)	4 (1.7)	3 (1.3)
Ecchymosis	0	3 (1.3)	3 (1.3)
Rectal hemorrhage	2 (0.8)	2 (0.8)	2 (0.8)
Hematuria	2 (0.8)	3 (1.3)	1 (0.4)
Vaginal hemorrhage	0	0	3 (1.3)
Gastrointestinal hemorrhage	1 (0.4)	1 (0.4)	1 (0.4)
Lower gastrointestinal hemorrhage	1 (0.4)	1 (0.4)	0
Cerebral hemorrhage	0	0	1 (0.4)
Diarrhea hemorrhagic	0	0	1 (0.4)
Gastritis hemorrhagic	0	0	1 (0.4)
Hematochezia	0	0	1 (0.4)
Hemorrhagic stroke	0	0	1 (0.4)
Mallory-Weiss syndrome	0	0	1 (0.4)

Table 6. Nonocular Hemorrhagic Adverse Events (cont'd)

MedDRA* Preferred Term	Sham (n = 236)	Ranibizumab 0.3 mg (n = 238)	Ranibizumab 0.5 mg (n = 239)
(cont'd) — n (%)			
Upper gastrointestinal hemorrhage	0	0	1 (0.4)
Hemoptysis	0	1 (0.4)	0
Hemorrhage	0	1 (0.4)	0
Lip hemorrhage	0	1 (0.4)	0
Melena	0	1 (0.4)	0
Periorbital hematoma	0	1 (0.4)	0
Subarachnoid hemorrhage	0	1 (0.4)	0
Subdural hemorrhage	0	1 (0.4)	0
Gastric ulcer hemorrhage	1 (0.4)	0	0
Hematotympanum	1 (0.4)	0	0
Hemorrhage urinary tract	1 (0.4)	0	0

*Medical Dictionary for Regulatory Activities.

† The number of patients with any nonocular hemorrhagic adverse event.

METHODS (online)

Study Design

MARINA was a 2-year, prospective, multicenter, randomized, double-masked, sham injection–controlled study of the safety, tolerability, and efficacy of repeated intravitreal injections of ranibizumab in patients with choroidal neovascularization due to neovascular AMD. The prespecified primary efficacy analysis was at 12 months. Institutional Review Board approval was obtained prior to patient enrollment, and HIPAA-compliance was achieved at each of the 96 study sites throughout the United States. Patients provided written, informed consent before determination of their full eligibility. The screening period could last up to 28 days.

Full patient eligibility criteria are available online (Table 1). The following are the major inclusion criteria: age at least 50 years old; best corrected visual acuity of 20/40 to 20/320 (Snellen equivalent) determined using a standardized refraction protocol with the Early Treatment Diabetic Retinopathy Study (ETDRS) charts; diagnosis of primary or recurrent choroidal neovascularization, secondary to AMD, involving the foveal center; lesion assessed by the investigator, using fluorescein angiography and fundus photography, as minimally classic (less than 50 percent of the lesion consisted of classic choroidal neovascularization) or occult with no classic choroidal neovascularization; maximum lesion size of 12 disc areas (DA), with the neovascular component comprising at least 50 percent of the entire lesion; and presumed recent disease progression evidenced by the presence of blood, recent vision loss, or a recent increase in a lesion's greatest linear diameter by 10 percent or more. The definition of a

lesion included the choroidal neovascularization and also blockage from hemorrhage, blocked fluorescence not from hemorrhage, pigment epithelial detachment, and fibrosis. Before enrollment, lesion eligibility was confirmed by an independent central reading center using trained masked graders and standardized criteria.

Major patient exclusion criteria were any prior treatment of the neovascular lesion (other than thermal laser sparing the foveal center), and treatment of the nonstudy eye with verteporfin photodynamic therapy less than 7 days before the first study treatment (day 0). There were no exclusion criteria regarding preexisting cardiovascular, cerebrovascular, or peripheral vascular conditions.

Study Treatment

Eligible patients were randomly assigned in a 1:1:1 ratio, using a dynamic randomization algorithm, to receive ranibizumab (LUCENTIS™, Genentech, Inc., South San Francisco, CA) 0.3 or 0.5 mg or a sham injection monthly (30 ± 7 days) for 2 years (24 injections). Randomization was stratified by baseline visual acuity score (< 55 letters [approximately worse than 20/80] vs. ≥ 55 letters) at day 0, by choroidal neovascularization subtype (minimally classic or occult with no classic), and by study center. Only one eye per patient received the study treatment. If both eyes were eligible, the eye with the better visual acuity was treated unless, for medical reasons, the investigator considered the other eye more appropriate.

Masking of treatment assignment required at least two investigators per study site: an evaluating physician (masked to treatment assignment), and an injecting physician (unmasked regarding ranibizumab or sham treatment but masked to ranibizumab dose). All other study site personnel (except those assisting with injections), patients, and central reading center personnel were masked to treatment assignment.

Following unmasking of first-year results and discussion with the Data Safety Monitoring Committee, the study protocol was amended in October 2005 to offer access to ranibizumab (0.5 mg) to patients in the sham group who had not completed the treatment period. Patients in the ranibizumab arms remained in their originally assigned treatment group. The study drug kits continued to be dispensed via the Interactive Voice Response System to maintain the masking of patients and all site personnel other than the injecting physician and those assisting in the injecting procedure.

Verteporfin photodynamic therapy for the study eye was allowed if the choroidal neovascularization in the study eye converted to a predominantly classic pattern. Following a national policy decision by the Centers for Medicare and Medicaid Services on April 1, 2004, to reimburse photodynamic therapy for small, minimally classic, and occult lesions, the study protocol was amended to allow photodynamic therapy for active minimally classic or occult with no classic lesions that were no larger than 4 DA in size and accompanied by a 20-letter or greater loss from baseline visual acuity

confirmed at consecutive study visits. When photodynamic therapy was used, the scheduled study treatment was held until the next scheduled monthly study visit.

For 3 days before and after each injection, patients self-administered prescribed topical antimicrobial drops to the study eye. On injection days, after antiseptic preparation of the eye and pretreatment with local anesthesia and antimicrobials, patients in the ranibizumab groups received their assigned dose in a 50- μ L solution administered as an intravitreal injection. Sham-treated patients underwent the same pre- and postinjection procedures, but ethical considerations precluded actual intravitreal injections. Instead, a sham injection was performed whereby pressure was applied to the eye at the site of a typical injection but using a syringe without a needle.

After injection, patients remained in the clinic at least 60 (\pm 10) minutes for additional safety monitoring and measurement of intraocular pressure. Site personnel contacted patients 2 (\pm 1) days postinjection to elicit reports of any new ocular symptoms in the study eye, and to inquire whether patients self-administered the prescribed antimicrobials. Patients were evaluated 7 days after their first ranibizumab or sham injection, but they did not return 7 days after subsequent injections.

Study Assessments

For the prespecified primary analysis of treatment efficacy, best corrected visual acuity and lesion characteristics at 12 months were compared with baseline. The primary efficacy end point was the proportion of patients losing fewer than 15 letters (approximately 3 lines) from baseline visual acuity, assessed with the ETDRS chart using a standardized refraction and testing protocol at a starting test distance of 2

meters. Other prespecified visual acuity efficacy end points included mean change over time, proportion of patients gaining 15 or more letters, and proportion of patients with a Snellen equivalent of 20/200 or worse. Exploratory visual acuity end points included the proportion of patients with a Snellen equivalent of 20/40 or better, the proportion of patients with a Snellen equivalent of 20/20 or better, and the proportion of patients losing 30 letters (approximately 6 lines) or more. Prespecified secondary end points involving lesion characteristics included mean change in the area of choroidal neovascularization and the area of leakage (including intense, progressive retinal pigment epithelium staining). To assess the long-term maintenance of treatment efficacy, the above end points were also evaluated at 24 months. Indirect ophthalmoscopy, intraocular pressure measurement, visual acuity testing, and slit lamp examination were performed by the evaluating physician before every monthly study treatment. Intraocular pressure was also measured at 60 minutes postinjection. Safety outcomes included incidence and severity of ocular and nonocular adverse events (see Tables 3-5 online for criteria and grading of ocular serious adverse events), changes and abnormalities in clinical laboratory parameters and vital signs, and assessment of immunoreactivity to ranibizumab. During the study, an independent data monitoring committee met twice per year to review unmasked safety summaries prepared by an external statistical coordinating center.

Analysis Methods

Efficacy analyses were performed on an intent-to-treat basis (all randomized patients) using a last observation carried forward method to handle missing data. For all pairwise comparisons, the statistical model stratified by baseline visual acuity score

(< 55 letters versus \geq 55 letters) and choroidal neovascularization subtype at baseline (minimally classic versus occult with no classic). Cochran χ^2 tests²¹ were used for between-group comparisons for dichotomous end points. Analysis of variance models were used to analyze change from baseline visual acuity. For lesion characteristic end points, analysis of covariance models that adjusted for baseline value were used. The Hochberg-Bonferroni multiple comparison procedure²² was used to adjust for the two pairwise treatment comparisons for the primary end point. Safety analyses included all patients who received at least one study treatment. All adverse event reports and safety assessments made after sham-treated patients crossed over to ranibizumab were excluded from the main analyses and evaluated separately.

Sample Size and Power Calculation

The sample size was determined based on the primary efficacy end point. Calculations were based on a 1:1:1 randomization ratio, the Pearson's χ^2 test for comparison of two proportions, and the Hochberg-Bonferroni multiple comparison procedure at an overall Type I error of 0.0497 (after adjusting for the three planned safety interim analyses prior to the primary efficacy analysis). Monte Carlo simulations were used to evaluate the power of the study. The planned sample size of 720 would have provided 95 percent power to detect a statistically significant difference between one or both ranibizumab groups and the sham control group in the proportion of patients losing fewer than 15 letters at 12 months, assuming a proportion of 65 percent in each ranibizumab group and 50 percent in the sham control group.