

Supplementary Appendix

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

Supplement to: Gragoudas ES, Adamis AP, Cunningham ET Jr, et al. Pegaptanib for neovascular age-related macular degeneration. *N Engl J Med* 2004;351:2805-16.

Methods

The study was designed by the V.I.S.I.O.N Steering Committee, the data were gathered and analyzed by the Data Management and Statistical Office, and the manuscript was written by the V.I.S.I.O.N. Writing Committee (please see appendix for detailed listing).

Study design

Two concurrent, prospective, randomized, double-masked, multicenter, dose-ranging, controlled clinical trials included patients at 117 sites in the United States, Canada, Europe, Israel, Australia, and South America. These studies were identically designed in order to fulfill the worldwide regulatory requirements of reaching statistical significance in two independent trials. Provided that both trials yielded significant treatment effects for the primary endpoint, the statistical analysis plan specified that the trials would be combined to produce more reliable secondary analyses. The studies were conducted in full conformance with the principles of the Declaration of Helsinki or with the laws of the country in which the research was conducted, whichever afforded the greater protection to the study participant. Institutional Review Board or Ethics Committee approval was obtained from each clinical center and signed informed consent was obtained from all study participants.

Patient selection

Patients aged ≥ 50 years with subfoveal choroidal neovascularization (CNV) secondary to age-related macular degeneration (AMD) and a best-corrected visual acuity in the study eye of 20/40 to 20/320 and in the fellow eye of 20/800 or better were eligible for inclusion. All angiographic subtypes were enrolled (predominantly classic [$\geq 50\%$ classic neovascularization comprising the lesion], minimally classic [1 to 49%], or occult with no classic [0%]), and total

lesion sizes up to and including 12 disc areas (including blood, scar/atrophy, and neovascularization) were permitted. No greater than 50% of the lesion could be comprised of subretinal hemorrhage, and $\geq 50\%$ of the lesion had to be CNV as defined previously.¹ Patients with minimally classic or occult with no classic CNV were required to have at least one of the following: subretinal hemorrhage associated with CNV, but comprising no more than 50% of the lesion; the presence of lipid; the loss of 15 or more letters (approximately 3 lines on the study eye chart) of visual acuity during the previous 12 weeks. Patients with a history of up to one photodynamic therapy (PDT) treatment were eligible only if the PDT treatment occurred between 8 and 13 weeks prior to the baseline visit. Other inclusion criteria were an intraocular pressure of ≤ 23 mm Hg, clear ocular media, and adequate pupillary dilation to permit good quality stereoscopic fundus photography.²

Patients were ineligible to participate in the study if they had atrophy exceeding 25% of the total lesion area or subfoveal scarring in the study eye. Patients with a history of previous subfoveal thermal laser therapy or previous or concomitant therapy with any investigational agent to treat AMD (except vitamins and minerals) were excluded. Other exclusion criteria were a likelihood of requiring cataract surgery within 2 years; other potential causes of CNV, including myopia of 8 diopters or more or axial length of 25 mm or more, ocular histoplasmosis syndrome, angioid streaks, choroidal rupture, or multifocal choroiditis; any intraocular surgery within 3 months or extrafoveal/juxtafoveal laser within 2 weeks of study entry; previous posterior vitrectomy or scleral buckling surgery; and presence of retinal pigment epithelial tears or rips. Patients also were excluded if they had diabetic retinopathy; history or evidence of severe cardiac disease (New York Heart Association functional class III or IV); a myocardial infarction within 6 months; ventricular tachyarrhythmia requiring ongoing treatment or unstable angina; a

history or evidence of peripheral vascular disease; stroke within 12 months of study entry; acute ocular or periocular infection; previous therapeutic radiation to the eye, head, or neck; any treatment with an investigational agent in the past 60 days for any condition; and known serious allergies to fluorescein dye (and indocyanine green, if used) or to components of the pegaptanib formulation.

An eligibility and classification quality assurance team with extensive experience in fluorescein angiographic interpretation of CNV¹ based at the Johns Hopkins University School of Medicine's Wilmer Ophthalmological Institute (Baltimore, MD) confirmed eligibility and angiographic lesion classification for stratification at randomization. Angiographic measurements of total lesion size, CNV size and leakage severity were performed in a masked fashion according to a standardized protocol at the University of Wisconsin Independent Fundus Photograph and Angiogram Reading Center.

Randomization and treatment

Patients were allocated in each trial to one of four treatment arms (sham or 0.3 mg, 1 mg, or 3 mg pegaptanib) by a dynamic procedure using a stochastic treatment allocation algorithm based on the variance method to minimize imbalances simultaneously for study center, angiographic lesion subtype and previous treatment with PDT.

Patients were assigned to receive either sham injection or intravitreal pegaptanib every 6 weeks for 48 weeks, for a total of 9 treatments. Pegaptanib, formulated for intravitreal injection at 0.3 mg/90 µL, 1 mg/90 µL, and 3 mg/90 µL concentrations in preservative-free, phosphate-buffered saline (pH 5 to 7), was packaged in a sterile, single-use, USP type 1 graduated glass 1 mL syringe with a preattached 27-gauge needle to minimize the risk of

contamination. All patients (including those receiving sham) underwent a common ocular antisepsis procedure and received injected subconjunctival anesthetic. To maintain patient masking, patients receiving sham were treated identically to patients receiving the study medication, with the exception of scleral penetration. Patients receiving sham had an identical but needleless syringe pressed against the eye wall to mimic active doses, which were injected via the pars plana into the vitreous cavity. The injection technique precluded the patient seeing the syringe. To maintain investigator masking, the study ophthalmologist responsible for patient care and assessments did not administer the injection. In all cases, a separate, certified visual acuity examiner masked to treatment assignment and to previous visual acuity measurements assessed distance visual acuity.

Due to ethical considerations, the use of PDT with verteporfin was permitted in all study arms within 5 to 10 days prior to each study treatment only for patients with predominantly classic lesions as defined in the Food and Drug Administration-approved product labeling and at the discretion of the masked ophthalmologist. The option for PDT administration represented an important feature of the study design, allowing pegaptanib efficacy to be tested against a background of usual care so that results could be extrapolated more reasonably to prevailing practice patterns.

Study assessments and endpoints

Efficacy

An ophthalmologic history and all baseline assessments were conducted within 7 days prior to the first study treatment. Certified examiners masked to treatment arm and to the results

of previous visual acuity measurements assessed best-corrected visual acuity at baseline and at every 6 week visit prior to study treatment.

The prespecified primary efficacy endpoint was the proportion of patients losing <15 letters of visual acuity (3 lines on the study eye chart) from baseline to Week 54. This endpoint was analyzed when all patients had reached Week 54, with no interim analysis of efficacy data. Fifteen letters on the study eye chart approximates a doubling of the visual angle, which is a clinically meaningful change for patients and an accepted regulatory endpoint. Patients were classified as Responders (those losing <15 letters of visual acuity, including those gaining visual acuity) or as Non-responders (those losing ≥ 15 letters of visual acuity from baseline). The null hypothesis stated that there was no difference in the primary efficacy endpoint between pegaptanib and sham. Additional efficacy endpoints included proportions of patients maintaining or gaining ≥ 0 , 5, 10, or 15 letters, losing ≥ 30 letters, mean changes in visual acuity at 6 week intervals from baseline to Week 54, and the proportion of patients having visual acuities of 20/200 or worse (legal blindness when involving the better seeing eye) at Week 54.

Safety

Intraocular pressure measurements and an ophthalmologic examination were performed at baseline and at every 6 week visit just prior to study treatment, at 30 minutes post-treatment and 1 week post-treatment. Vital signs were recorded and routine hematological and biochemical analyses were conducted at baseline and at every 6 week visit prior to treatment. Color fundus photography and fluorescein angiography were performed at baseline and at Weeks 30 and 54 prior to treatment and were examined by the University of Wisconsin reading center for unexpected alterations in retinal anatomy.

Final study assessments were performed on all patients who withdrew, and those who withdrew due to an adverse event were followed until the adverse event resolved or an adequate explanation for the event was obtained. Safety endpoints included all adverse events and serious adverse events whether or not they were deemed related to the drug or to the injection procedure, as well as any laboratory test abnormalities. An Independent Data Monitoring and Safety Monitoring Committee consisting of experts independent from the sponsor and the investigators reviewed the data and procedures of both trials on an ongoing basis to assure patient safety.

Statistical analyses

Sample size calculation

Sample size calculations were based on the proportion of patients losing ≥ 15 letters of visual acuity at 54 weeks, which was estimated to be 50% for the sham group based on historical data.³ Assuming that the two-sided significance level of the analysis was set at 5%, and that treatment reduced the proportion of patients losing ≥ 15 letters of visual acuity at 54 weeks by 20%, 122 patients were required for each treatment arm to provide an overall power of 95%. Assuming that 10% of all patients would be ineligible or yield data that could not be evaluated, 135 patients per group were recruited and randomized in each study.

Data analysis

Because the two studies were identical in design and similar in baseline characteristics, data from the two studies were pooled for presentation. The prespecified primary efficacy analyses were performed on all randomized patients who received at least one study treatment and had baseline visual acuity assessments. For all efficacy analyses, patients were evaluated in the treatment group to which they were randomly assigned. Several analyses of the primary

efficacy endpoint that accounted for missing data were also conducted. Prespecified safety analyses included all patients who received at least one study treatment, irrespective of whether a baseline visual acuity was obtained.

Analyses of binary endpoints were based on proportions of patients in each treatment group using the last observation carried forward to impute missing data. A prespecified Cochran-Mantel-Haenszel (CMH) test using stratification factors (lesion angiographic subtype, prior PDT), baseline visual acuity (≥ 54 letter visual acuity score vs. < 54 letter visual acuity score; 54 letters equals approximately 20/80 on the Snellen eye chart), and baseline lesion size (< 4 disc areas vs. ≥ 4 disc areas) was applied to comparisons of binary endpoints. In addition, a CMH test on proportions using only observed data was carried out to assess the robustness of the results to the imputation of missing data. Mean changes in visual acuity were analyzed using an analysis of covariance model and observed mean changes for each time point; models included main effects for treatment and stratification factors, with baseline visual acuity and baseline lesion size (both treated as continuous variables) as covariates. To control for multiple doses, a prespecified Hochberg multiple comparison procedure⁴ was used to compare each dose in a pairwise fashion against sham and to control the two-sided experiment-wise type I error rate at 5% in each of the two trials. All p-values reported in the present paper are 2-sided and unadjusted for multiplicity. In order to study the potential influence of patient characteristics on treatment efficacy, mean change in visual acuity from baseline to Week 54 was compared between pegaptanib and sham. Patient characteristics of interest were prespecified in the statistical analysis plan and included baseline angiographic lesion subtype (predominantly classic, minimally classic, and occult), baseline visual acuity (≥ 54 letter visual acuity score, < 54 letter visual acuity score), and baseline lesion size (< 4 disc areas, ≥ 4 disc areas). In addition, multiple logistic regression analyses using

backwards selection with alpha at 0.05 to identify main effects or interactions between treatment and potentially relevant patient characteristics were performed in a pairwise fashion. The factors studied included age, gender, baseline lesion composition, baseline lesion size, baseline visual acuity, fellow eye status, smoking status, lipid, more than 3 lines of recent visual acuity loss, race and prior/baseline PDT use.

References

1. Macular Photocoagulation Study Group. Subfoveal neovascular lesions in age-related macular degeneration: Guidelines for evaluation and treatment in the Macular Photocoagulation Study. *Arch Ophthalmol* 1991;109:1242-57.
2. Allen L. Ocular fundus photography: Suggestions for achieving consistently good pictures and instructions for stereoscopic photography. *Am J Ophthalmol*;1964;57:13-28.
3. Pharmacological Therapy for Macular Degeneration Study Group. Interferon alfa-2a is ineffective for patients with choroidal neovascularization secondary to age-related macular degeneration. Results of a prospective randomized placebo-controlled clinical trial. *Arch Ophthalmol* 1997;115:865-72.
4. Hochberg Y. A sharper Bonferroni procedure for multiple tests of significance. *Biometrika* 1988;75:800-2.