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EVALUATION OF FULL-FIELD ELECTRORETINOGRAM REDUCTIONS AFTER OCRIPLASMIN TREATMENT

Results of the OASIS Trial ERG Substudy

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Purpose: To explore a possible association between full-field electroretinograms with vitreomacular adhesion resolution and best-corrected visual acuity as part of the prospective, randomized, double-masked, sham-controlled Ocriplasmin for Treatment for Symptomatic Vitreomacular Adhesion Including Macular Hole (OASIS) trial studying ocriplasmin.

Methods: The ERG substudy enrolled 62 of 220 OASIS subjects (randomized 2:1) and analyzed full-field electroretinograms and their association with both vitreomacular adhesion resolution and best-corrected visual acuity from baseline through Month 24. Electroretinogram reductions were defined as acute full-field electroretinogram reductions in amplitude of ≥40% from baseline occurring at postinjection Day 7 or Day 28.

Results: In the ocriplasmin group, 16/40 (40%) subjects developed ERG reductions, compared to 1/21 (4.8%) in the sham group; 13/16 (81.3%) and 1/1 (100%) resolved by study end, respectively. A total of 11/16 (68.8%) ocriplasmin-treated subjects with ERG reductions achieved vitreomacular adhesion resolution, compared to those without (9/24, 37.5%). The ocriplasmin-treated subjects with ERG reductions also gained more letters on average (11.3 vs. 9.3 letters) from baseline and had a difference of 6.7 letters in mean best-corrected visual acuity by study end compared to those without ERG reductions.

Conclusion: Ocriplasmin-treated subjects with ERG reductions had a higher rate of vitreomacular adhesion resolution and showed better visual improvement than their counterparts without ERG reductions or sham subjects by study end.

RETINA 38:364–378, 2018

Following the pivotal Phase 3 clinical trials (NCT00781859 and NCT00798317) for ocriplasmin, a pharmacological treatment of vitreomacular traction (VMT; also referred to as symptomatic vitreomacular adhesion [VMA]), multiple studies were initiated to enhance the understanding of ocriplasmin efficacy and safety. Subsequent studies focused on longer follow-up times, real-world settings, and specific safety profiles. The Ocriplasmin for Treatment for Symptomatic Vitreomacular Adhesion Including Macular Hole (OASIS) study is a Phase 3b clinical trial designed to characterize ocriplasmin efficacy and safety over a 24-month follow-up period (compared to a 6-month follow-up in earlier trials) in a patient population with specific characteristics shown to be associated with higher rate of VMA resolution. As part of the larger OASIS trial, a full-field electroretinogram (ffERG) substudy was initiated. The purpose of the substudy was to specifically investigate a possible association between amplitude reductions in ffERG recordings (hereafter referred to as ERG reductions) and both VMA resolution and changes in visual acuity with respect to ocriplasmin treatment. The substudy was also to assess whether ERG reductions represented relevant clinical events.

Electroretinograms were not regularly obtained in the ocriplasmin clinical trial program, making the incidence of ERG reductions after ocriplasmin treatment in Phase 2 and Phase 3 trials difficult to calculate.[1] In early Phase 2 trials (TG-MV-001 [NCT00123279] and TG-MV-002 [NCT00412451]), nine of the collective 98 patients exposed to

ocriplasmin were reported to have ERG reductions.[1] However, for these trials, there was no standardized ERG technology for the type of instruments used across the sites, no certification was required, and no central reading center (CRC) was involved for the ERG recording. In addition, most recordings from TG-MV-001 were obtained after vitrectomy. These cases were not reported as adverse events (AEs), but were evaluated by the FDA during the market authorization process.[1] No safety signal was associated with treatment-related reductions in ERG amplitude.

In subsequent clinical trials, 10 cases of ERG reductions were reported (9 cases from TG-MV-008 [Phase 2]; 1 case from TG-MV-007 [Phase 3]). The noted ERG reductions included decreases in the a- and b-wave amplitudes, but no responses were isoelectric.[1] Beyond the clinical trials, abnormal ERG events related to ocriplasmin have been continually monitored from postmarketing sources. As of October 16, 2015, there have been 29 cases of retinogram abnormal event reported from various postmarketing sources per 23,330 vials of ocriplasmin distributed, representing a cumulative reporting rate of 1.25 per 1,000 doses. The low postmarketing frequency could be due to ERG recordings not being typically collected in a clinical setting.

In the past several years, individual case reports have also been published on abnormal ERG readings in certain patients after ocriplasmin treatment.[2–4] In most cases, however, failure to obtain an ERG recording before ocriplasmin injection confounds the interpretation of these results. Without a baseline value, it is not possible to determine whether an ERG abnormality was present prior to ocriplasmin treatment or was due to nonspecific effects of the drug. In addition, ERG recordings can exhibit wide variation;[5] therefore, only reductions of ≥40% are considered abnormal and clinically significant for the International Society for Clinical Electrophysiology of Vision (ISCEV) protocol,[6] and by the FDA, which uses the ISCEV guidelines.[5–10]

To our knowledge, baseline ERG recordings have not been previously systematically examined in patients with VMA. The purpose of the ERG substudy was to provide standardized analyses not possible with individual case reports, as part of a larger prospective, double-masked, sham-controlled, multicenter clinical trial. This standardization included use of the same type of instrument and recording protocol, as well as certification of instruments and personnel and a masked CRC. In addition, the ERG substudy offered other benefits over case reports, such as baseline recordings, a patient population studied concurrently, a long-term follow-up period, and determination of possible associations of ffERG reductions with both anatomic (i.e., VMA resolution on optical coherence tomography) and functional assessments (i.e., visual acuity).

Methods

The OASIS study (TG-MV-014, NCT01429441) was a Phase 3b, randomized, sham-controlled, double-masked, multicenter clinical trial designed to further evaluate the long-term efficacy and safety of ocriplasmin in subjects with symptomatic VMA/VMT. Subjects were randomized to receive either a single intravitreal injection of ocriplasmin 0.125 mg or sham treatment. Full details of the OASIS trial methods and results are to be published elsewhere.

As part of the larger study, an ERG substudy was initiated involving a subset of the clinical study sites. The goal of the substudy was to explore the relationship between ffERG and both VMA resolution and best-corrected visual acuity (BCVA) from baseline through Month 24. A total of 62 subjects from the OASIS study (N = 220) were enrolled in the ERG substudy (41 of 146 [28.1%] ocriplasmin, 21 of 74 [28.4%] sham). One subject in the ocriplasmin group had a baseline but no postinjection ERG assessment and therefore was not included in the ERG subset.

There were 12 study visits performed as follows: baseline, Day 0 (injection day), Day 7 (±2 days), Day 28 (±3 days), Month 3 (±7 days), and every 3 months (±14 days) thereafter until Month 24.

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Financial support for this article was provided by ThromboGenics, Iselin, NJ. The sponsor or funding organization participated in the design of the study, conducting the study, data collection, data management, data analysis, interpretation of the data, preparation, and review or approval of the manuscript.

Some of this work has been presented at the American Academy of Ophthalmology Annual Meeting, Las Vegas, NV, November 14–17, 2015, and the Association for Research in Vision and Ophthalmology Annual Meeting, Seattle, WA, May 1–5, 2016.

D. G. Birch reports consulting fees from ThromboGenics; D. M. Miller reports consulting fees, grants, and payment for lectures from ThromboGenics, outside of the submitted work; A. N. Antoszyk reports consulting fees from ThromboGenics; J. Markoff reports consulting fees from ThromboGenics; P. Kozma is an employee of ThromboGenics; E. Meunier is a consultant for ThromboGenics; R. C. Sergott reports consulting fees, travel support, and fees for participation in review activities from ThromboGenics. The remaining author has no conflicting interests to disclose.

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Full-field ERGs were performed in both eyes at each visit, including the baseline visit, with the exception of the injection day visit.

Full-field ERG assessments were performed by certified study personnel at selected sites. In total, six sites were certified to perform the ERG evaluations. The Espion E^2 console and a full-field ColorDome (Diagnosys LLC, Lowell, MA) were used following a standard setup at each of these sites for the ffERG assessment. Full-field ERGs were completed only in subjects enrolled in the substudy. The recordings were evaluated by the masked CRC for ERGs.

For the ERG procedure, the ISCEV protocol was followed. The eyes were dark adapted and the pupils dilated with standard dilating drops at the site before recording. Recordings were obtained with a Dawson Trick Litzkow fiber electrode on the surface of the eye along the lower eyelid, in contact with the tear film and cul de sac.

"Expert-defined ERG reductions" were specified as those ffERG reductions from baseline that were considered relevant by the masked ERG expert assessor based on the ffERG responses only, without consideration of any other visual function, and were identified before unmasking. The judgment to determine the presence of a relevant ERG reduction for a subject at a visit was based on the number and combination of the responses at the visit that were reduced by ≥40%. The threshold for significant reduction (with 95% confidence interval) is fairly similar among ISCEV standard responses.[10] For a decrease in amplitude at 95% confidence interval, the threshold for the rod response has been reported as 40% (for patients with retinitis pigmentosa and cone-rod dystrophy)[6] or 41% (for patients without diffuse photoreceptor cell disease).[5] The comparable threshold for a decrease in light-adapted 30 Hz flicker amplitude has been reported as 35%,[9] 37%,[6] or 52%.[5] Thus, considering a 40% average reduction in amplitude across the ISCEV standard responses as meaningful is reasonable based on the literature. Only reductions ≥40% are considered abnormal by the ISCEV.[7] The FDA also uses this level as a guideline.[7,8] The reductions occurring just after injection (Day 7 or Day 28 visits) were of particular interest due to a possible association with treatment and are referred to as "acute expert-defined ERG reductions." All descriptions of ERG reductions in the article are considered acute and "expert-defined" and will be hereafter referred to as "ERG reductions." Change from baseline in rod response, combined rod–cone response a-wave, combined rod–cone response b-wave, cone response, 30 Hz flicker, and combined rod–cone response peak-to-peak amplitude were assessed throughout the study. The assessment of the ERG reductions was based mainly on the change from baseline in amplitude, although prolonged implicit times and shifts in implicit times from baseline were also part of the evaluation.

After unmasking, associations between ERG measurements and visual function measurements were examined, including BCVA, Amsler grid evaluation, Pelli-Robson contrast sensitivity score, Roth 28-hue color vision assessment, and the National Eye Institute 25-item Visual Function Questionnaire (VFQ). Analyses for associations between ERG measurements and anatomical measurements were performed, including central retinal thickness, ellipsoid zone (EZ) in the central 1 mm cube, external limiting membrane in the central 1 mm cube, subretinal fluid, and photoreceptor area and thickness measurements.

The ERG subset was the set of all subjects who were enrolled at selected sites, and for whom measurements of ffERG were available pre- and post-injection. Analyses were based on actual treatment received. No formal statistical testing was performed. Descriptive statistics for continuous variables included number of subjects with available data, mean and SD, and where appropriate, median. Categorical data were summarized based on counts, percentages, and 95% confidence intervals for the proportion (Clopper–Pearson method), where appropriate.

Results

Demographics and Baseline Ocular Characteristics

Demographics and baseline characteristics for subjects in the ERG subset are shown in Table 1. Demographics and baseline characteristics in the ERG subset were comparable between the ocriplasmin and the sham group, as well as to the overall OASIS subject population.

In the ERG subset, baseline ocular characteristics were largely comparable between the ocriplasmin and sham groups (Table 2). In the ocriplasmin group, the time since VMA diagnosis was longer than 12 months in a higher percentage of subjects compared with the sham group (6/40, 15% vs. 0/21, 0%). Of those with macular hole at baseline, there was a higher percentage of subjects in the ocriplasmin group with small (≤250 μm) macular hole (8/16, 50%) compared with the sham group (3/10, 30%), which had a higher percentage of midsize (>250–400 μm) macular hole (5/10, 50%) compared with the ocriplasmin group (5/16, 31.3%).

Baseline ERG characteristics for the study eye of subjects in the ERG subset were comparable between the treatment groups (Table 3). A total of 12/61 (19.7%) subjects presented abnormal

Table 1. Demographics and Baseline Characteristics of the ERG Substudy and Overall OASIS Trial

Demographics and Baseline Characteristics	Sham (N = 21)	Ocriplasmin (N = 40)	Overall ERG Substudy (N = 61)	Overall OASIS Trial (N = 220)
Sex, n (%)				
Male	6 (28.6)	14 (35.0)	20 (32.8)	72 (32.7)
Female	15 (71.4)	26 (65.0)	41 (67.2)	148 (67.3)
Race, n (%)				
White	19 (90.5)	36 (90.0)	55 (90.2)	197 (89.5)
Black	2 (9.5)	4 (10.0)	6 (9.8)	18 (8.2)
Other	NA	NA	NA	7 (3.3)
Ethnicity, n (%)				
Non-Hispanic	20 (95.2)	39 (97.5)	59 (96.7)	204 (92.7)
Hispanic	1 (4.8)	1 (2.5)	2 (3.3)	16 (7.3)
Age (years) at baseline				
Mean (SD)	67.9 (10.6)	69.2 (9.2)	68.7 (9.6)	69.1 (10.30)
Median	70.0	68.0	68.0	68.0
Min, Max	39, 87	53, 90	39, 90	38, 94

NA, not applicable.

amplitudes at baseline as assessed by the masked CRC for ERGs (especially for cones at baseline taking age into account). The median reduction in amplitude in these patients from the reference value was 32% for the cone response and 57% for the rod response. Of these subjects, six were in the ocriplasmin group and six in the sham group. Only two of these subjects experienced ERG reductions after ocriplasmin treatment.

Baseline characteristics were also compared for subjects with and without ERG reductions regardless of the treatment group. Fewer subjects with ERG reductions had epiretinal membrane at baseline compared to those without ERG reductions. The proportion of subjects with VMA diagnosis greater than 12 months and the mean VMA diameter at baseline was higher in subjects without ERG reductions (Table 4). There were slightly more subjects with ERG reductions that had likely/definitive sites of incomplete foveal EZ compared to those without (70.6% vs. 63.6%, respectively), but not enough to clearly establish a trend (Table 4). Similarly, there was a trend toward more subjects with ERG reductions having subretinal fluid at baseline compared to those without ERG reductions (82.4% vs. 65.9%, respectively). Electroretinogram amplitudes at baseline were similar between those who experienced ERG reductions compared to those who did not (Table 4).

Resolution of Electroretinogram Reductions

A total of 16/40 (40%) subjects in the ocriplasmin group experienced an episode of ERG reduction in the study eye, compared with 1/21 (4.8%) subjects in the sham group (Figure 1). Both a- and b-waves were affected. In the nonstudy eye, ERG reductions occurred in 3/61 (4.9%) subjects overall. Electroretinogram reductions resolved in most cases. In the ocriplasmin group, 13/16 (81.3%) cases and 1/1 (100%) cases in the sham group resolved by study end. In the ocriplasmin group, 9/12 (75%) cases that began by the Day 7 visit resolved, with a median time to resolution of 176 days, and 4/4 (100%) that began after Day 7 and before the Day 28 visit resolved, with a median time to resolution of 68 days. In the sham group, 1 case that began after Day 7 and before the Day 28 visit resolved in 66 days (Figure 1). Electroretinogram resolution was defined as within 40% of baseline.

Subjects With Unresolved Electroretinogram Reductions

Three subjects in the ocriplasmin group experienced ERG reductions that did not resolve by study end. Subject 1 achieved VMA resolution and had isoelectric ERGs by the Day 7 visit. This subject had a macular hole of >400 μm at baseline per the CRC assessment and therefore was not eligible for study participation. This subject subsequently had a vitrectomy 18 days after treatment and cataract removal. Scalloping can be seen on the optical coherence tomography 10 days after vitrectomy, and although the macular architecture looks sound, the ERG responses were still flat (Figure 2A, middle panels). By the Month 24 visit, the ERG recordings showed signs of improvement in the rod–cone response, the cone-dominated response, and the cone-isolated response (Figure 2A, lower panels). At study end, the subject gained 13 letters from baseline and showed improved VFQ scores (26.7 points for composite and

Table 2. Baseline Ocular Characteristics of the OASIS ERG Substudy

Baseline Ocular Characteristics (ERG Substudy)	Sham (N = 21)	Ocriplasmin (N = 40)	Overall ERG Substudy (N = 61)	Overall OASIS Trial (N = 220)
VMA at baseline (based on SD-OCT), n (%)				
Present	21 (100)	39 (97.5)	60 (98.4)	213 (96.8)
Absent	0	1 (2.5)	1 (1.6)	7 (3.2)
Diameter of VMA at baseline (based on SD-OCT), n (%)				
≤1,500 μm	19 (90.5)	37 (92.5)	56 (91.8)	192 (87.3)
>1,500 μm	2 (9.5)	1 (2.5)	3 (4.9)	16 (7.3)
Missing	0	2 (5.0)	2 (3.3)	12 (5.5)
FTMH at baseline (based on SD-OCT), n (%)				
Present	10 (47.6)	16 (40.0)	26 (42.6)	76 (34.5)
Absent	11 (52.4)	24 (60.0)	35 (57.4)	144 (65.5)
Largest of the minimum macular hole width (based on SD-OCT), n (%)				
≤250 μm	3 (30.0)	8 (50.0)	11 (42.3)	34 (44.7)
>250–400 μm	5 (50.0)	5 (31.3)	10 (38.5)	28 (36.8)
>400 μm	2 (20.0)	3 (18.8)	5 (19.2)	14 (18.4)
ERM at baseline (based on SD-OCT), n (%)				
Present	5 (23.8)	9 (22.5)	14 (23.0)	51 (23.2)
Absent	16 (76.2)	31 (77.5)	47 (77.0)	169 (76.8)
Lens status, n (%)				
Phakic	17 (81.0)	30 (75.0)	47 (77.0)	158 (71.8)
Pseudophakic	4 (19.0)	10 (25.0)	14 (23.0)	62 (28.2)
Central retinal thickness (based on SD-OCT), μm				
Mean (SD)	211.3 (196.1)	277.1 (270.1)	254.4 (247.5)	231.3 (202.33)
Median	94.0	187.5	139.0	189.0
Subretinal fluid (based on SD-OCT), n (%)				
No	7 (33.3)	11 (27.5)	18 (29.5)	84 (38.2)
Yes	14 (66.7)	29 (72.5)	43 (70.5)	136 (61.8)
BCVA (ETDRS letter score)				
Mean (SD)	62.0 (11.5)	64.6 (8.2)	63.7 (9.4)	63.2 (9.65)
Snellen	20/63	20/50	20/50	20/50
Median	65.0	65.5	65.0	65.0
Snellen	20/50	20/50	20/50	20/50
Time since VMA diagnosis (months), n (%)				
<1	6 (28.6)	10 (25.0)	16 (26.2)	69 (31.4)
1–3	11 (52.4)	23 (57.5)	34 (55.7)	96 (43.6)
4–6	4 (19.0)	1 (2.5)	5 (8.2)	15 (6.8)
7–12	0	0	0	10 (4.5)
13–24	0	3 (7.5)	3 (4.9)	17 (7.7)
>24	0	3 (7.5)	3 (4.9)	13 (5.9)
EZ in the central 1 mm cube, n (%)				
Definitely fully intact	6 (28.6)	9 (22.5)	15 (24.6)	92 (41.8)
Likely site(s) of incomplete EZ, foveal	0	4 (10.0)	4 (6.6)	4 (1.8)
Likely site(s) of incomplete EZ, nonfoveal	0	1 (2.5)	1 (1.6)	5 (2.3)
Definite site(s) of incomplete EZ, foveal	14 (66.7)	22 (55.0)	36 (59.0)	105 (47.7)
Definite site(s) of incomplete EZ, nonfoveal	1 (4.8)	2 (5.0)	3 (4.9)	6 (2.7)
Unable to grade	0	2 (5.0)	2 (3.3)	7 (3.2)
Missing	0	0	0	1 (0.5)

All SD-OCT assessments were determined by masked CRC.

ERM, epiretinal membrane; ETDRS, Early Treatment Diabetic Retinopathy Study; FTMH, full-thickness macular hole; SD-OCT, spectral domain optical coherence tomography.

40.0 points for general vision), indicating that cone function was partially spared.

Subject 2 did not achieve VMA resolution. This subject did not have a macular hole at baseline, but an unspecified foveal red lesion was reported. This subject showed a markedly reduced b-wave amplitude at the baseline visit (although not enough to be categorized as abnormal at baseline), which persisted at the Day 7 visit after ocriplasmin injection (Figure 2B). At the Month 24 visit, both the a- and b-wave amplitudes

Table 3. Baseline ERG Characteristics of the OASIS ERG Substudy

Baseline ERG Characteristics (ERG Substudy)	Sham (N = 21)	Ocriplasmin (N = 40)	Overall ERG Substudy (N = 61)	Nonstudy Eye (N = 61)
Rod response amplitude, μV				
N	18	36	54	53
Mean (SD)	263.5 (110.5)	231.8 (113.8)	242.4 (112.7)	240.2 (109.4)
Combined rod–cone response a-wave amplitude, μV				
n	21	38	59	60
Mean (SD)	214.8 (42.0)	223.5 (75.5)	220.4 (65.2)	219.5 (68.7)
Combined rod–cone response b-wave amplitude, μV				
N	21	38	59	60
Mean (SD)	158.6 (92.6)	170.2 (84.0)	166.1 (86.5)	169.1 (87.8)
Combined rod–cone response peak-to-peak amplitude, μV				
N	21	38	59	60
Mean (SD)	373.1 (97.3)	393.7 (115.2)	386.3 (108.8)	388.5 (113.9)
Cone response amplitude, μV				
N	21	38	59	60
Mean (SD)	122.7 (42.7)	114.8 (43.3)	117.6 (42.9)	114.5 (40.9)
30 Hz flicker amplitude, μV				
N	21	40	61	61
Mean (SD)	103.9 (39.8)	99.3 (38.0)	100.9 (38.3)	100.9 (34.6)

were significantly reduced. By study end, this subject maintained visual acuity (gained 2 letters from baseline) and showed a 1.9-point decrease in the composite (82.5–80.6) and maintenance of the general vision VFQ scores from baseline (60.0).

Subject 3 had a macular hole at baseline of 270 μm (max–min by CRC), with macular hole worsening (defined as an increase of $\geq$50 μm from baseline) that was noted 6 days after injection. This subject achieved VMA resolution by the Day 7 visit and subsequently required multiple surgeries after ocriplasmin injection: two vitrectomies for macular hole closure, cataract surgery, and subsequent surgery to remove silicone oil. This subject lost 14 letters by study end; however, VFQ results showed maintenance in the composite score (81.0–81.5) and an improvement in the general vision score from baseline (60.0–80.0).

Detailed Sample Cases of Subjects With Electroretinogram Reductions

Individual cases from among the substudy subjects highlight the variation in ERG responses independent of and following ocriplasmin treatment. More typical

Table 4. Baseline Characteristics in Subjects With and Without ERG Reductions

Baseline Characteristics	With ERG Reductions (N = 17)	Without ERG Reductions (N = 44)
ERM at baseline	1 (5.9)	13 (29.6)
Phakic lens status	16 (94.1)	13 (29.5)
VMA diagnosis >12 months, n (%)	1 (5.9)	5 (11.3)
Diameter of focal VMA at baseline (μm), mean (SD)	400.2 (195.0)	574.9 (449.6)
Likely/definite sites of incomplete foveal EZ, n (%)	11 (64.7)	25 (56.8)
Subretinal fluid, n (%)	14 (82.4)	29 (65.9)
Thin retinal thickness	11 (64.7)	25 (56.8)
Rod response amplitude (μV), mean (SD)	269.2 (96.0)	230.0 (118.8)
Combined rod–cone response a-wave amplitude (μV), mean (SD)	241.7 (65.5)	211.8 (63.9)
Combined rod–cone response b-wave amplitude (μV), mean (SD)	159.1 (97.2)	168.9 (82.9)
Combined rod–cone response peak-to-peak amplitude (μV), mean (SD)	400.8 (118.9)	380.5 (105.3)
Cone response amplitude (μV), mean (SD)	125.9 (39.1)	114.2 (44.3)
30 Hz flicker amplitude (μV), mean (SD)	102.8 (37.7)	100.1 (39.0)

ERM, epiretinal membrane.

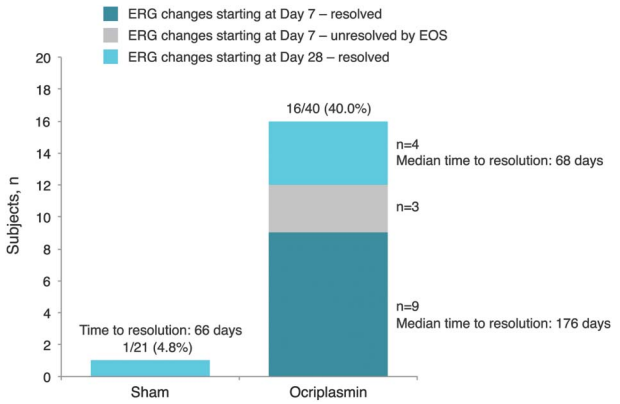

Fig. 1. Resolution of ERG reductions in the ocriplasmin and sham groups. Acute "expert-defined" ERG reductions were defined as a ≥40% change in amplitude from baseline observed at either Day 7 or Day 28 visit in a set of ERG recordings considered relevant by the masked ERG expert assessor, without consideration of any other visual function. In the sham group, one subject experienced ERG reductions, which resolved by study end. In the ocriplasmin group, 16 subjects experienced ERG reductions, 13 of which resolved by study end. N = 40 ocriplasmin, N = 21 sham. EOS, end of study.

were transient ERG reductions after ocriplasmin treatment that went on to resolve (Figure 3A). One subject showed VMA resolution by the Day 7 visit, and significant scotopic loss at the Day 28 visit as evidenced by reduction in the rod response and combined rod–cone response amplitudes (Figure 3A, middle panels). However, at the Month 24 visit, all ERGs were within the normal range, with the rod response and combined rod–cone b-wave amplitudes higher than baseline (Figure 3A, lower panels).

Other ocriplasmin-treated subjects showed no subsequent ERG abnormalities compared with baseline (Figure 3B). One subject showed ERG responses within the normal range at both the Day 7 and Day 28 visits (Figure 3B, middle and lower panels), which are the visits that would define ERG reductions related to ocriplasmin treatment. Interestingly, one-fifth of the study participants from both the ocriplasmin and sham treatment groups had abnormal ERGs at baseline. One sham subject showed reduced rod response amplitudes at baseline (Figure 3C). Furthermore, this amplitude and combined rod-cone a- and b-wave amplitudes, cone response amplitude, and 30 Hz flicker amplitude were all lower at the Day 28 visit compared with baseline (Figure 3C, upper and lower panels). Such ERG abnormalities are independent of ocriplasmin and are likely due to tractional forces.

Vitreomacular Adhesion Resolution and Electroretinogram Reductions

Determination of VMA resolution after ERG reductions was one of the main objectives of the ERG substudy. In the ocriplasmin group, 10/16 (62.5%) subjects with ERG reductions achieved pharmacological VMA resolution by Day 28; 7/16 (43.8%) achieved VMA resolution by Day 7 (Figure 4). For those without ERG reductions in the ocriplasmin group, 7/24 (29.2%) subjects achieved VMA resolution by Day 28, and 3/24 (12.5%) showed VMA resolution by Day 7 (Figure 4). In the sham group, incidences of VMA resolution were lower. A total of 2/20 (10%) subjects without ERG reductions achieved VMA release by Day 7, with no further subjects achieving VMA release by Day 28. The sham subject with ERG reductions did not achieve VMA resolution by Day 28.

Visual Acuity

Assessment of visual acuity was another objective of the ERG substudy. Importantly, in the ocriplasmin group, 15/16 (93.8%) subjects with ERG reductions showed improvement in BCVA (>0 Early Treatment Diabetic Retinopathy Study [ETDRS] letters) from baseline to study end, compared with 18/24 (75.0%) of those without ERG reductions. All but two ocriplasmin subjects with ERG reductions gained at least one line, with 10 gaining at least two lines and five gaining at least three lines. One ocriplasmin subject with ERG reductions showed a decline of at least two lines. The ocriplasmin-treated subjects with ERG reductions had an improvement of 6.7 letters in mean BCVA by study end compared to those without ERG reductions (78.4 letters [20/32] vs. 71.7 letters [20/40], respectively) (Figure 5). In the sham group, 13/20 (65.0%) subjects without ERG reductions showed BCVA improvement >0 letters, compared with 0/1 (0%) subjects with ERG reductions. Four sham subjects showed a BCVA decline of at least one line. However, there were no linear correlations found between BCVA and ERG amplitudes.

Visual Function Measurements and Electroretinogram Reductions

A number of other visual function measurements were also assessed for association with ERG measurements, including Amsler grid evaluation, Pelli-Robson contrast sensitivity score, and Roth 28-hue color vision assessment. Of these, only the color vision assessment showed an association with ERG reductions; mean ERG amplitudes tended to be consistently higher in subjects without color vision defect compared to those with defect. Ocriplasmin-treated subjects with ERG reductions tended to experience a shift from no color vision defect at baseline to defect more often than those without ERG reductions. A total of 6/16 (37.5%)

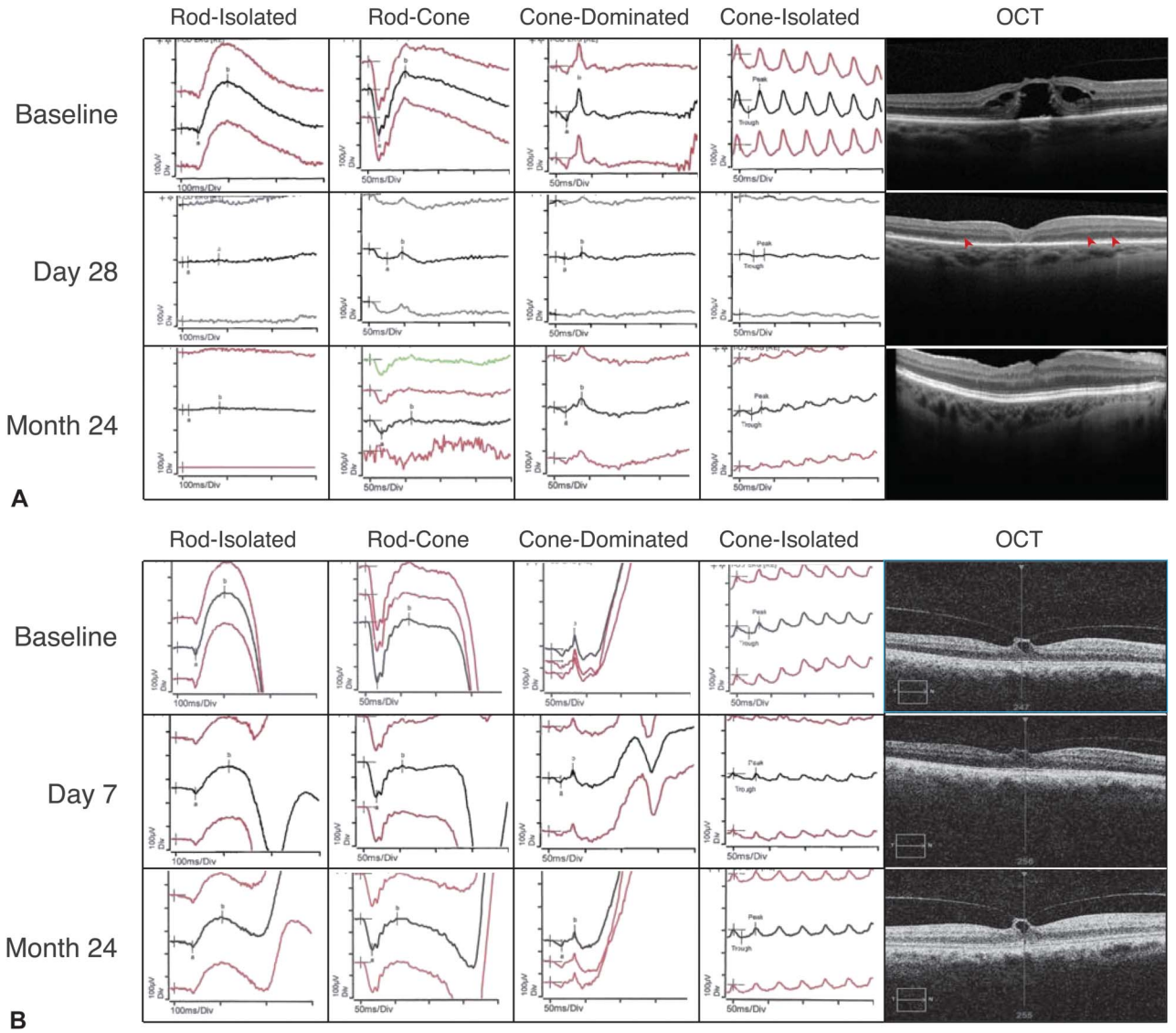

Fig. 2. Two cases of unresolved ERG reductions by study end. **A.** Subject 1 with unresolved ERG reductions by study end. Study eye is OD. Selected ERG recordings per study visit are as indicated. Baseline: dark-adapted 0.01 ERG (rod response) amplitude, 204.3 μV; dark-adapted 3.0 (combined rod–cone response) a-wave amplitude, 206.2 μV; b-wave amplitude, 83.3 μV; light-adapted 3.0 ERG (cone response) amplitude, 143.6 μV; light-adapted 3.0 flicker (30 Hz flicker) amplitude, 101.7 μV. Day 28: dark-adapted 0.01 ERG (rod response) amplitude, 12.3 μV; dark-adapted 3.0 (combined rod–cone response) a-wave amplitude, 47.6 μV; b-wave amplitude, 16.7 μV; light-adapted 3.0 ERG (cone response) amplitude, 32.2 μV; light-adapted 3.0 flicker (30 Hz flicker) amplitude, 9.0 μV. Month 24: dark-adapted 0.01 ERG (rod response) amplitude, 11.4 μV; dark-adapted 3.0 (combined rod–cone response) a-wave amplitude, 59.2 μV; b-wave amplitude, 11.9 μV; light-adapted 3.0 ERG (cone response) amplitude, 64.3 μV; light-adapted 3.0 flicker (30 Hz flicker) amplitude, 35.7 μV. Scalloping is observed on the OCT at Day 28 (arrowheads). **B.** Subject 2 with unresolved ERG reductions by study end. Study eye is OD. Selected ERG recordings per study visit are as indicated. Baseline: dark-adapted 0.01 ERG (rod response) amplitude, 247.3 μV; dark-adapted 3.0 (combined rod–cone response) a-wave amplitude, 274.8 μV; b-wave amplitude, 14.2 μV; light-adapted 3.0 ERG (cone response) amplitude, 94.3 μV; light-adapted 3.0 flicker (30 Hz flicker) amplitude, 53.6 μV. Day 7: dark-adapted 0.01 ERG (rod response) amplitude, 92.6 μV; dark-adapted 3.0 (combined rod–cone response) a-wave amplitude, 152.4 μV; b-wave amplitude, 16.9 μV; light-adapted 3.0 ERG (cone response) amplitude, 56.5 μV; light-adapted 3.0 flicker (30 Hz flicker) amplitude, 22.8 μV. Month 24: dark-adapted 0.01 ERG (rod response) amplitude, 135.0 μV; dark-adapted 3.0 (combined rod-cone response) a-wave amplitude, 143.4 μV; b-wave amplitude, 2.6 μV; light-adapted 3.0 ERG (cone response) amplitude, 65.3 μV; light-adapted 3.0 flicker (30 Hz flicker) amplitude, 46.3 μV. OCT, optical coherence tomography; OD, right eye.

versus 2/20 (10%) experienced a shift at Day 7 and 3/14 (21.4%) versus 1/19 (5.3%) at Day 28 for ocriplasmin subjects with and without ERG reductions, respectively. These values were comparable by study end for these two groups, with 2/14 (14.3%) versus 3/20 (15%) experiencing a shift in color vision from normal to abnormal. This compares to 2/17 (11.8%) of sham subjects without ERG reductions experiencing a shift in color vision at Day 28 and study end, respectively.

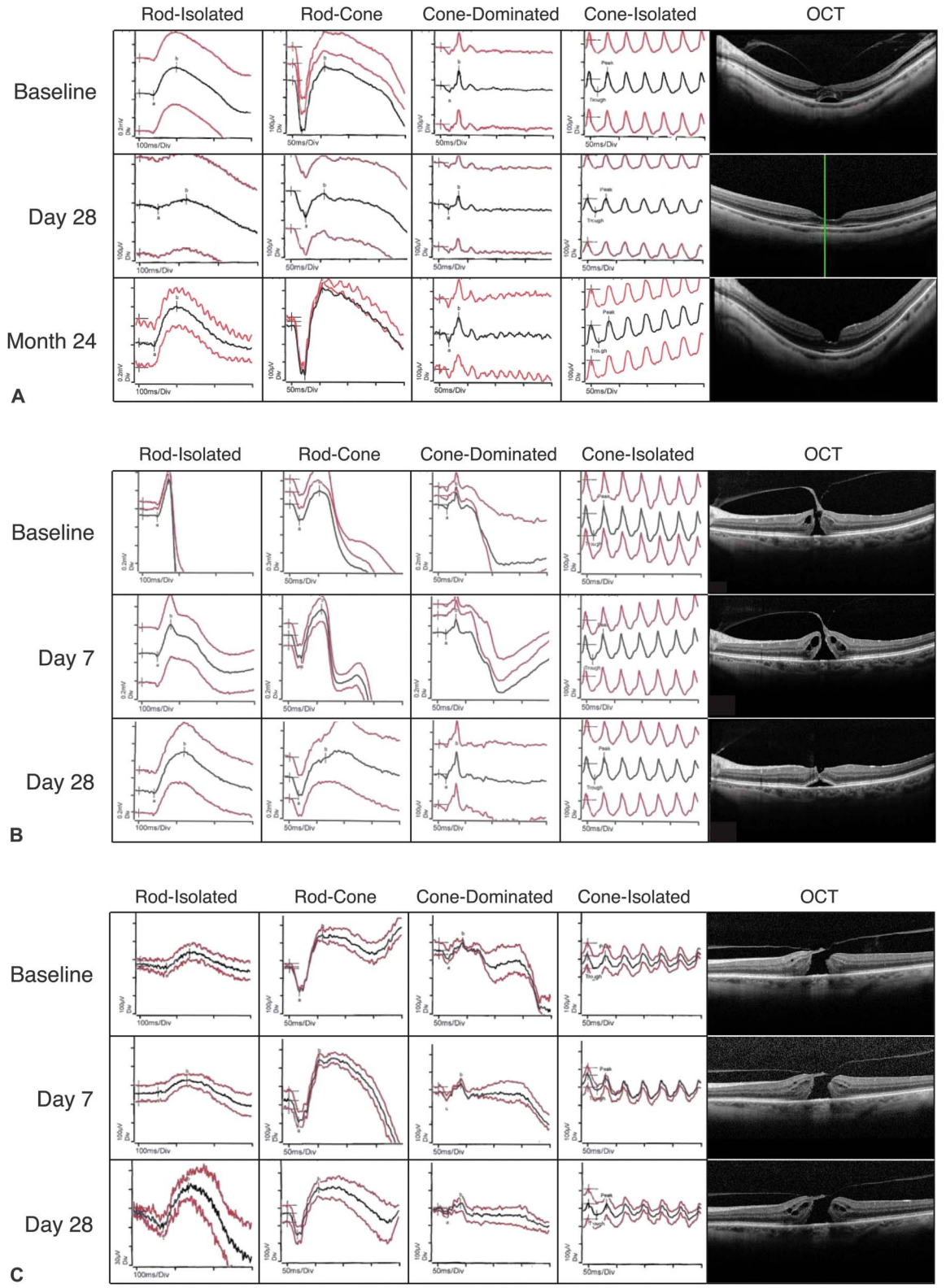

Fig. 3. Case examples of subjects with various ERG baselines and outcomes from the substudy. **A.** Patient experiencing ERG reductions after ocriplasmin treatment with subsequent recovery. Study eye is OS. Baseline: dark-adapted 0.01 ERG (rod response) amplitude, 287.2 μV; dark-adapted 3.0 (combined rod–cone response) a-wave amplitude, 290.5 μV; b-wave amplitude, 78.4 μV; light-adapted 3.0 ERG (cone response) amplitude, 122.4 μV; light-adapted 3.0 flicker (30 Hz flicker) amplitude, 115.0 μV. Day 28: dark-adapted 0.01 ERG (rod response) amplitude, 30.4 μV; dark-adapted 3.0 (combined rod–cone response) a-wave amplitude, 142.0 μV; b-wave amplitude, 16.7 μV; light-adapted 3.0 ERG (cone response) amplitude, 77.9 μV;

For Amsler grid, because of the large number of subjects with an abnormal status at baseline, it was not possible to determine the association between shift from baseline for Amsler grid assessment and ERG amplitude. Most subjects in both treatment groups had an abnormal Amsler grid evaluation at baseline (38/40 [95.0%] subjects in the ocriplasmin group and 18/21 [85.7%] subjects in the sham group). For contrast sensitivity, the proportion of subjects experiencing a decline in contrast sensitivity score (defined as a shift from $\geq$1.5 to $<$1.5) from Day 7 to Month 6 tended to be greater for subjects with ERG reductions than for those without, although the number of subjects was small (n = 12 with and n = 10 without ERG reductions). However, the linear correlation coefficients suggest that there was no linear association between the contrast sensitivity score and ERG reductions.

Anatomical Measurements and Electroretinogram Reductions

The anatomical measurements described at baseline, namely incomplete foveal EZ, presence of subretinal fluid, and photoreceptor area and thickness measurements were analyzed for correlations with ERG amplitudes. There were no correlations between ERG amplitudes and subjects with either intact or incomplete EZ. Similar to EZ changes, there were no linear correlations between photoreceptor area and thickness measurements and ERG amplitudes. Although there was a trend toward a higher proportion of subjects with ERG reductions having subretinal fluid, there was no clear link between the presence or absence of subretinal fluid and ERG reductions.

Subjects in the ocriplasmin group with ERG reductions tended to have thinner mean central subfields at baseline compared with ocriplasmin-treated subjects without ERG reductions, and the thicker retinas of those without ERG reductions were borderline normal or fell outside of the normal range (173.6 [166.5] μm vs. 346.1 [305.3] μm, respectively). Thickness measurements were comparable between the two groups at the Month 9 visit. No linear correlations were observed between ERG amplitudes and central retinal thickness.

Safety

The proportion of subjects in the ocriplasmin group who reported ocular AEs in the study eye was comparable between those with and without ERG reductions (15/16, 93.8% vs. 23/24, 95.8%), but greater than the proportion of subjects in the sham group without ERG reductions (17/20, 85.0%). The most commonly reported AEs in the study eye of ocriplasmin-treated subjects with ERG reductions included vitreous floaters, photopsia, macular hole, and visual acuity reduced. For ocriplasmin-treated subjects without ERG reductions, the most commonly reported AEs in the study eye were vitreous floaters, nuclear cataract photopsia, and macular hole. For sham subjects without ERG reductions, the most commonly reported AEs in the study eye were visual acuity reduced, cataract, photopsia, and macular hole (Table 5). In the ocriplasmin group, the proportion of subjects who reported serious AEs in the study eye was greater in those with ERG reductions versus those without (5/16, 31.3% vs. 5/24, 20.8%), and comparable with the proportion of subjects in the sham group without ERG reductions (6/20, 30%). Adverse events of special interest (defined as significant AEs, consisting of a group of predefined MedDRA terms that are of particular clinical importance, other than serious AEs and those leading to discontinuation of the subject from the study) were reported more frequently in subjects in the

light-adapted 3.0 flicker (30 Hz flicker) amplitude, 75.9 μV. Month 24: dark-adapted 0.01 ERG (rod response) amplitude, 384.3 μV; dark-adapted 3.0 (combined rod–cone response) a-wave amplitude, 277.1 μV; b-wave amplitude, 214.4 μV; light-adapted 3.0 ERG (cone response) amplitude, 140.3 μV; light-adapted 3.0 flicker (30 Hz flicker) amplitude, 121.2 μV. **B.** Patient with no ERG reductions after ocriplasmin treatment. Study eye is OS. Selected ERG recordings per study visit are as indicated. Baseline: dark-adapted 0.01 ERG (rod response) amplitude, 402.6 μV; dark-adapted 3.0 (combined rod–cone response) a-wave amplitude, 278.6 μV; b-wave amplitude, 222.8 μV; light-adapted 3.0 ERG (cone response) amplitude, 195.4 μV; light-adapted 3.0 flicker (30 Hz flicker) amplitude, 169.6 μV. Day 7: dark-adapted 0.01 ERG (rod response) amplitude, 316.6 μV; dark-adapted 3.0 (combined rod–cone response) a-wave amplitude, 252.2 μV; b-wave amplitude, 288.9 μV; light-adapted 3.0 ERG (cone response) amplitude, 171.1 μV; light-adapted 3.0 flicker (30 Hz flicker) amplitude, 147.5 μV. Day 28: vitreomacular traction observed at the Day 28 visit. Dark-adapted 0.01 ERG (rod response) amplitude, 461.7 μV; dark-adapted 3.0 (combined rod–cone response) a-wave amplitude, 204.7 μV; b-wave amplitude, 229.9 μV; light-adapted 3.0 ERG (cone response) amplitude, 160.2 μV; light-adapted 3.0 flicker (30 Hz flicker) amplitude, 129.4 μV. **C.** Patient with abnormal ERGs at baseline. Study eye is OS. Selected ERG recordings per study visit are as indicated. Baseline: dark-adapted 0.01 ERG (rod response) amplitude, 82.0 μV; dark-adapted 3.0 (combined rod–cone response) a-wave amplitude, 142.4 μV; b-wave amplitude, 182.1 μV; light-adapted 3.0 ERG (cone response) amplitude, 95.6 μV; light-adapted 3.0 flicker (30 Hz flicker) amplitude, 72.8 μV. Day 7: dark-adapted 0.01 ERG (rod response) amplitude, 74.8 μV; dark-adapted 3.0 (combined rod–cone response) a-wave amplitude, 116.3 μV; b-wave amplitude, 223.9 μV; light-adapted 3.0 ERG (cone response) amplitude, 101.2 μV; light-adapted 3.0 flicker (30 Hz flicker) amplitude, 69.5 μV. Day 28: dark-adapted 0.01 ERG (rod response) amplitude, 63.3 μV; dark-adapted 3.0 (combined rod–cone response) a-wave amplitude, 124.5 μV; b-wave amplitude, 147.4 μV; light-adapted 3.0 ERG (cone response) amplitude, 65.5 μV; light-adapted 3.0 flicker (30 Hz flicker) amplitude, 63.4 μV. OCT, optical coherence tomography; OS, left eye.

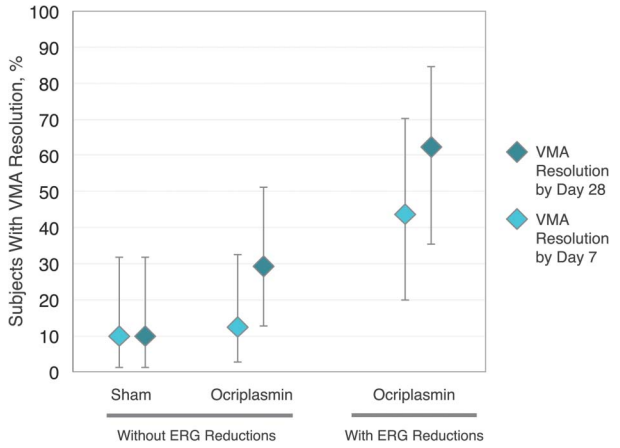

Fig. 4. Pharmacological VMA resolution by Day 7 and Day 28 by ERG reductions in the ocriplasmin and sham groups. Proportion of subjects with VMA resolution based on the presence or absence of ERG reductions. Subjects were assessed for VMA resolution without anatomical defect at Day 7 and Day 28 visits. Analysis was performed with postresolution vitrectomy considered as a failure, using LOCF as the imputation method. The n values for Day 28 represent the total number of subjects achieving VMA resolution by Day 28 (primary end point). VMA status was assessed at all visits using SD-OCT. N = 24, ocriplasmin subjects without ERG reductions; N = 16, ocriplasmin subjects with ERG reductions; N = 20, sham subjects without ERG reductions; 1 sham subject with ERG reductions is not shown. Error bars represent 95% confidence interval. LOCF, last observation carried forward; SD-OCT, spectral domain optical coherence tomography.

ocriplasmin group with ERG reductions compared to those without, including visual alteration (11/16, 68.8% vs. 13/24, 54.2%), dyschromatopsia (9/16, 56.3% vs. 10/24, 41.7%), eye pain (5/16, 31.3% vs. 2/24, 8.3%), and subretinal fluid (3/16, 18.8% vs. 2/24, 8.3%). Overall, AEs of special interest occurred in 13/16 (81.3%) subjects in the ocriplasmin group with ERG reductions, in 20/24 (83.3%) subjects in this group without ERG reductions, and in 16/20 (80.0%) subjects in the sham group without ERG reductions.

Discussion

The ERG substudy of the OASIS clinical trial is the first study where the effect of ocriplasmin on ffERGs was assessed in a standardized, systematic manner as part of a prospective, randomized, double-masked, sham-controlled, multicenter clinical trial over a 24-month period. Because prospective ERG recordings are not typically performed clinically, only 1 case report has been published to date with baseline ERGs for comparison to ERGs taken in response to visual symptoms associated with ocriplasmin treatment.[4] The prospective nature of the ERG substudy allowed for standardization and comparison of changes to baseline—analyses not possible with retrospective cases.

A total of 40% of the subjects treated with ocriplasmin experienced ERG reductions. In addition, 20% of overall subjects who were part of both treatment groups presented with abnormal ERGs at baseline, suggesting in both cases a panretinal phenomenon in these eyes. Both a- and b-waves were affected. The relatively high number of subjects with abnormal baseline ERGs underscores the need for pretreatment recordings. Only three subjects had ERG reductions that did not resolve by study end. Despite this, only one of these subjects experienced a loss of visual acuity (this subject also

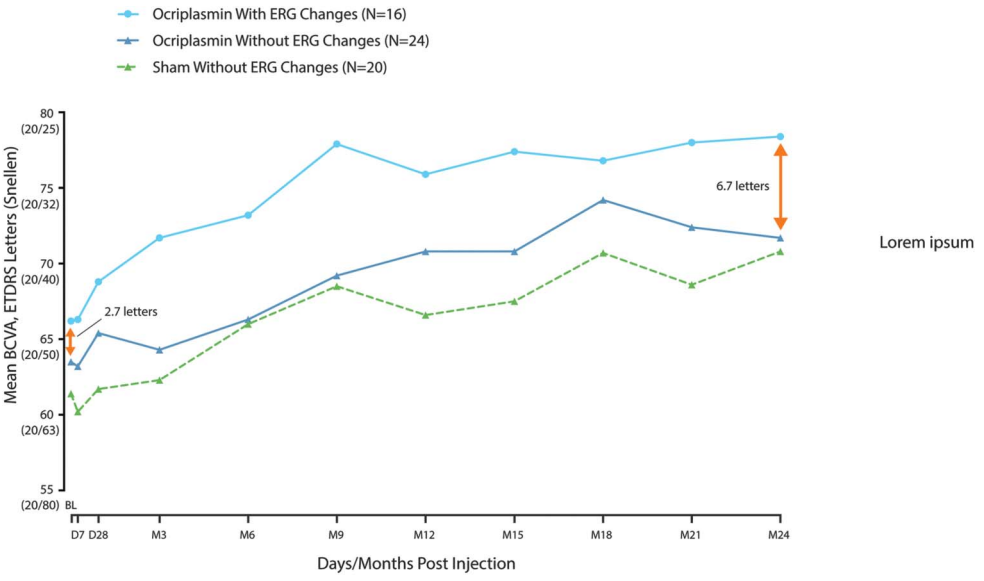

Fig. 5. Mean BCVA over time by ERG reductions in the ocriplasmin and sham groups. Mean BCVA at each study visit in ocriplasmin-treated subjects with and without ERG reductions and sham-treated subjects without ERG reductions. BCVA was reported as the number of letters read correctly on an ETDRS letter chart. Analysis was performed irrespective of vitrectomy, using LOCF as the imputation method. N = 24, ocriplasmin subjects without ERG reductions; N = 16, ocriplasmin subjects with ERG reductions; N = 20, sham subjects without ERG reductions. BL, baseline; D, day; ETDRS, Early Treatment Diabetic Retinopathy Study; LOCF, last observation carried forward; M, month.

Table 5. Summary of AEs Presented by ERG Reductions in the OASIS ERG Substudy

	Ocriplasmin With ERG Reductions (N = 16)			Ocriplasmin Without ERG Reductions (N = 24)			Sham Without ERG Reductions (N = 20)		
	n (%)	Events	95% CI	n (%)	Events	95% CI	n (%)	Events	95% CI
AEs									
Any event	16 (100)	191	79.4–100	23 (95.8)	159	78.9–99.9	19 (95.0)	121	75.1–99.9
Any nonocular event	12 (75.0)	52	47.6–92.7	14 (58.3)	30	36.6–77.9	15 (75.0)	50	50.9–91.3
Any ocular event	16 (100)	139	79.4–100	23 (95.8)	129	78.9–99.9	18 (90.0)	71	68.3–98.8
Any study eye event	15 (93.8)	125	69.8–99.8	23 (95.8)	114	78.9–99.9	17 (85.0)	64	62.1–96.8
Any nonstudy eye event	7 (43.8)	14	19.8–70.1	8 (33.3)	5	15.6–55.3	6 (30.0)	7	11.9–54.3
Preferred term									
Vitreous floaters	8 (50.0)	12	24.7–75.3	7 (29.2)	8	12.6–51.1	2 (10.0)	2	1.2–31.7
Photopsia	7 (43.8)	8	19.8–70.1	4 (16.7)	5	4.7–37.4	3 (15.0)	4	3.2–37.9
Macular hole	4 (25.0)	6	7.3–52.4	4 (16.7)	4	4.7–37.4	3 (15.0)	4	5.7–43.7
Posterior capsule opacification	4 (25.0)	4	7.3–52.4	3 (12.5)	3	2.7–32.4	2 (10.0)	2	1.2–31.7
Visual acuity reduced	4 (25.0)	4	7.3–52.4	2 (8.3)	2	1.0–27.0	6 (30.0)	7	11.9–54.3
Visual impairment	4 (25.0)	8	7.3–52.4	4 (16.7)	4	4.7–37.4	0	0	0.0–16.8
Nuclear cataract	3 (18.8)	3	4.0–45.6	5 (20.8)	6	7.1–42.2	3 (15.0)	4	3.2–37.9
Chromatopsia	3 (18.8)	3	4.0–45.6	2 (8.3)	2	1.0–27.0	0	0	0.0–16.8
Eye pain	3 (18.8)	3	4.0–45.6	0	0	0.0–14.2	1 (5.0)	1	0.1–24.9
Macular fibrosis	3 (18.8)	3	4.0–45.6	2 (8.3)	2	1.0–27.0	1 (5.0)	1	0.1–24.9
Photophobia	3 (18.8)	3	4.0–45.6	1 (4.2)	1	0.1–21.1	0	0	0.0–16.8
Subretinal fluid	3 (18.8)	3	4.0–45.6	2 (8.3)	2	1.0–27.0	3 (15.0)	3	3.2–37.9
Cataract	2 (12.5)	2	1.6–38.3	3 (12.5)	3	2.7–32.4	4 (20.0)	4	5.7–43.7
Conjunctival hyperemia	2 (12.5)	2	1.6–38.3	0	0	0.0–14.2	0	0	0.0–16.8
Lacrimation increased	2 (12.5)	2	1.6–38.3	1 (4.2)	1	0.1–21.1	0	0	0.0–16.8
Ocular discomfort	2 (12.5)	3	1.6–38.3	2 (8.3)	2	1.0–27.0	2 (10.0)	2	1.2–31.7
Ocular hypertension	2 (12.5)	2	1.6–38.3	0	0	0.0–14.2	0	0	0.0–16.8
Pupillary reflex impaired	2 (12.5)	2	1.6–38.3	0	0	0.0–14.2	0	0	0.0–16.8
Vision blurred	2 (12.5)	2	1.6–38.3	3 (12.5)	3	2.7–32.4	1 (5.0)	1	0.1–24.9

Results presented are events occurring in two or more subjects in the “ocriplasmin with ERG reductions” group. Table is sorted by this group in descending order.
CI, confidence interval.