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Single-Chain Antibody Fragment VEGF Inhibitor RTH258 for Neovascular Age-Related Macular Degeneration

A Randomized Controlled Study

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Purpose: To assess the safety and efficacy of different doses of RTH258 applied as single intravitreal administration compared with ranibizumab 0.5 mg in patients with neovascular age-related macular degeneration (AMD).

Design: Six-month, phase 1/2, prospective, multicenter, double-masked, randomized, ascending single-dose, active-controlled, parallel-group study.

Participants: A total of 194 treatment-naive patients, aged ≥50 years, with primary subfoveal choroidal neovascularization secondary to AMD.

Methods: Patients received a single intravitreal injection of RTH258 0.5 mg (n = 11), 3.0 mg (n = 31), 4.5 mg (n = 47), or 6.0 mg (n = 44), or ranibizumab 0.5 mg (n = 61).

Main Outcome Measures: The primary efficacy end point was the change from baseline to month 1 in central subfield thickness (CSFT) measured by spectral-domain optical coherence tomography. The secondary efficacy end point was the duration of treatment effect measured as time from the initial injection to receipt of post-baseline therapy (PBT) guided by protocol-defined criteria. Adverse events (AEs) were recorded throughout the study.

Results: RTH258 demonstrated noninferiority compared with ranibizumab in mean change in CSFT from baseline to month 1 for the 4.5- and 6.0-mg dose groups (margin: 40 μm, 1-sided alpha 0.05). The difference in CSFT change at month 1 comparison with ranibizumab was 22.86 μm (90% confidence interval [CI], −9.28 to 54.99) and 19.40 μm (95% CI, −9.00 to 47.80) for RTH258 4.5 and 6 mg, respectively. The median time to PBT after baseline therapy was 60 and 75 days for patients in the RTH258 4.5- and 6.0-mg groups, respectively, compared with 45 days for ranibizumab. Changes in best-corrected visual acuity with RTH258 were comparable to those observed with ranibizumab. The most frequent AEs reported for the RTH258 groups were conjunctival hemorrhage, eye pain, and conjunctival hyperemia; the majority of these events were mild in intensity.

Conclusions: This first-in-human study of RTH258 demonstrated noninferiority in the change in CSFT at 1 month for the 4.5- and 6.0-mg doses compared with ranibizumab and an increase of 30 days in the median time to PBT for the 6.0-mg dose. There were no unexpected safety concerns, and the results support the continued development of RTH258 for the treatment of neovascular AMD. *Ophthalmology 2016;123:1080-1089 © 2016 by the American Academy of Ophthalmology. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).*

Supplemental material is available at www.aaojournal.org.

Macular diseases, notably age-related macular degeneration (AMD), are leading causes of irreversible blindness and visual impairment. The 2010 global population statistical data indicate that 1 in 15 blind people and 1 in 32 visually impaired people had macular disease.[1] In addition, the rate of blindness due to macular diseases increased by 36% from 1990 to 2010.[1]

Anti−vascular endothelial growth factor (VEGF) therapies and novel imaging techniques, including spectral-domain optical coherence tomography (SD OCT), have contributed to remarkable improvements in early diagnosis, monitoring, and functional outcomes for patients with neovascular AMD[2] leading to decreasing or at least stable prevalence of blindness and visual impairment associated

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with macular disease in developed regions.[1,3,4] Current anti-VEGF therapies delivered via intravitreal injection include ranibizumab and aflibercept, as well as off-label bevacizumab,[5] and national and international guidelines recommend these anti-VEGF agents as first-line therapy for the treatment of neovascular AMD.[5,6] Research is ongoing regarding other molecular targets in the AMD disease pathway, including platelet-derived growth factor, mammalian target of rapamycin, several complement components, and combination therapies to improve the treatment of wet and dry AMD.[7]

Although anti-VEGF therapies have resulted in improved patient outcomes, there are limitations associated with these treatments. Anti-VEGF therapies require frequent clinical assessment to monitor patient response to treatment and, in some cases, monthly or bimonthly intravitreal injections.[2,7] The burden associated with frequent clinic visits or intravitreal injections for patients, caregivers, and physicians is significant and may lead to suboptimal outcomes because of adherence problems and underdosing.[2] LUMINOUS, an ongoing 5-year prospective, multinational, observational study designed to evaluate the outcomes of treatment with ranibizumab 0.5 mg in routine clinical practice, showed that patients with newly diagnosed neovascular AMD have an average of 7.5 visits and 4.7 injections in the first year of therapy, resulting in a gain of 4.4 letters. Over 2 years, the average number of visits was 13.6 with 8.7 injections and a gain of 2 letters.[8] Thus, there is a logistical disconnect between the realities of community practice and the strict monthly-visit regimen of well-known clinical trial schedules.[9–12] The AURA (A retrospective noninterventional study to assess the effectiveness of existing Anti-vascUlar endothelial growth factor [anti-VEGF] treatment Regimens in patients with wet Age-related macular degeneration) study, a retrospective analysis of 2227 real-world ranibizumab-treated patients with wet AMD who were followed for 2 years, reported a good initial response to therapy, with improved visual acuity over the first 120 days.[13] However, the average gains in visual acuity were not maintained over the 2-year follow-up.[13] Intravitreal injections declined from a mean of 5.0 injections in the first year to 2.2 injections in the second year. There was also a decline in the number of visits to the treating ophthalmologist over the same time period.[13] Alternative treatments or long-acting drug delivery systems would be required to offset the compromise between treatment burden and visual outcomes by increasing the drug efficacy and durability of treatment response.[7,14]

RTH258 (formerly ESBA1008) is a humanized single-chain antibody fragment that inhibits all isoforms of VEGF-A. It is the smallest anti-VEGF inhibitor tested in humans with a molecular weight of only 26 kDa compared with 48 kDa for ranibizumab or 115 kDa for aflibercept.[15] Because of its high stability and solubility, it is possible to concentrate RTH258 up to 120 mg/ml, allowing the administration of 6 mg in a single 50-μl intravitreal injection.[15] This enables the delivery of a much higher molar dose in the same volume as the current VEGF inhibitors in clinical use,[15,16] potentially supporting an early initiation and a prolonged duration of treatment effect. Furthermore, animal studies have shown that the small size of RTH258 leads to a fast systemic clearance and a 4-fold lower systemic exposure[15,16] compared with anti-VEGF agents like ranibizumab and bevacizumab, potentially reducing the risk of systemic side effects. The smaller size also may allow for better ocular tissue penetration.[16] The tolerability of high doses of RTH258 in animals, the high affinity to VEGF, and the lower molecular weight suggest that RTH258 may be an effective ocular anti-VEGF therapy in humans, with extended duration of efficacy.

The objective of this first-in-human study was to assess the safety, tolerability, and effect of treatment on ocular outcomes after a single intravitreal administration of 1 of 4 dose levels of RTH258 compared with ranibizumab 0.5 mg in patients with neovascular AMD.

Methods

Study Design

This phase 1/2, 6-month, prospective, multicenter, double-masked, randomized, ascending single-dose, active-controlled, parallel-group study compared the safety and efficacy of a single intravitreal injection of RTH258 (ascending doses) or ranibizumab as therapy for treatment-naive patients with neovascular AMD. The study protocol was approved by all institutional review boards and complied with the ethical standards defined by the Declaration of Helsinki and Good Clinical Practice. All patients provided written informed consent before participating in the study (ClinicalTrials.gov identifier, NCT01304693).

The study was conducted in 2 phases: (1) a dose escalation phase of RTH258 to the maximum feasible dose (MFD) for intravitreal injection (MFD = 6.0 mg RTH258) and (2) an MFD expansion phase. In the dose escalation phase, patients were randomized 5:2 to receive RTH258 (0.5, 3.0, or 4.5 mg) or ranibizumab 0.5 mg. Each dose level cohort was reviewed by a safety committee before the next ascending dose level cohort was treated and evaluated. Details regarding the safety review decision points for the ascending doses and the stopping criteria for the study are reported in the Appendix (available at www.aaojournal.org). Within each dose level cohort, the first 4 patients enrolled and treated with a single dose were sequentially assessed with a waiting period of ≥24 hours between patients to allow sufficient time for safety review for each patient before approving injection of the next patient. After the first 4 patients in a given dosing cohort received treatment and were reviewed for safety, the remaining patients in the cohort were treated in parallel.

The MFD expansion phase of the study consisted of 2 parts. In the first part, patients were randomized 1:1 to RTH258 4.5 mg or ranibizumab 0.5 mg. In the second part, patients were enrolled and randomized 5:30:35:9 to RTH258 0.5-, 3.0-, or 6.0-mg doses, or ranibizumab 0.5 mg. The randomization schedule was determined in part by additional data requirements for separate pharmacokinetic/pharmacodynamic studies related to RTH258.

All randomized patients were evaluated for safety and efficacy over 13 study visits, including screening, day of treatment, and 11 post-treatment follow-up visits (day 1, weeks 1 and 2, and months 1, 1.5, 2, 2.5, 3, 4, 5, and 6). Eligible patients were randomized using an interactive web response system to receive a single intravitreal injection of their assigned study drug in the study eye on day 0. Details regarding the randomization process are provided in the Appendix (available at www.aaojournal.org). The dose,

Table 1. Dose, Volume, and Concentration of Study Drug

Study Drug and Dose, mg	Volume Delivered, ml	Concentration, mg/ml
RTH258, 0.5	0.05	10
RTH258, 3.0	0.05	60
RTH258, 4.5	0.075	60
RTH258, 6.0	0.10	60
Ranibizumab, 0.5	0.05	10

injection volume, and concentration for the RTH258 0.5-, 3.0-, 4.5-, and 6.0-mg doses and the ranibizumab 0.5-mg dose are shown in Table 1.

At screening, all patients underwent best-corrected visual acuity (BCVA) assessments for both eyes, bilateral ophthalmic examinations, color fundus photography, fluorescein angiography, and measurements of central subfield thickness (CSFT) by SD OCT. The CSFT was measured for both eyes at screening and for the study eye at all post-screening study visits using an SD OCT instrument (SPECTRALIS; Heidelberg Engineering, Heidelberg, Germany). Retinal images obtained at screening were sent to a central reading center for the determination of patient eligibility on the basis of the lesion attributes specified in the inclusion criteria. Eligible and randomized patients received a single intravitreal injection of the study drug. All patients were monitored post-injection for optic nerve head perfusion and health of the eye until the central retinal artery was adequately perfused. Intraocular pressure in the study eye was monitored until the pressure was <30 mmHg and considered stable by the study investigator. Patients were monitored for adverse events (AEs) and instructed to seek immediate care from an ophthalmologist if the study eye became red, sensitive to light, or painful, or if there was a change in vision.

Patients

Treatment-naive patients, aged ≥50 years, presenting with primary subfoveal choroidal neovascularization secondary to AMD, including predominantly classic, minimally classic, or occult lesions at screening, were eligible to participate in the study. The study enrolled patients at 51 sites in the United States, Europe, Israel, and Australia. The study was conducted from October 2010 to March 2013. Details concerning the inclusion and exclusion criteria are provided in Table 2 (available at www.aaojournal.org).

Outcomes

The primary efficacy end point was the change from baseline to month 1 in CSFT as measured by SD OCT. The secondary efficacy end point was the duration of treatment effect measured by the time from initial treatment to the receipt of post-baseline therapy (PBT) as decided by the study investigator guided by protocol-specified criteria. Additional supportive analyses were conducted for the change from baseline in CSFT and BCVA at all time points in the study. Visual acuity was assessed using the Electronic Early Treatment Diabetic Retinopathy Study chart on an electronic tester at 3 m and was calculated as the number of letters read correctly plus 5 letters for each logarithm of the minimum angle of resolution line above the upper logarithm of the minimum angle of resolution level through 20/800.

Post-baseline therapy for exudative AMD was recommended on the basis of the following criteria: (1) beginning on day 14 if, in the opinion of the investigator, there was a decrease in the change from baseline in CSFT of <50 μm at any visit, a new intraocular hemorrhage deemed clinically significant, or vision loss of ≥7 letters of BCVA associated with an accumulation of retinal fluid compared with baseline; (2) beginning with month 1 if, in the opinion of the investigator, there was a ≥340-μm CSFT on SD OCT (based on the value specified for initial treatment in the

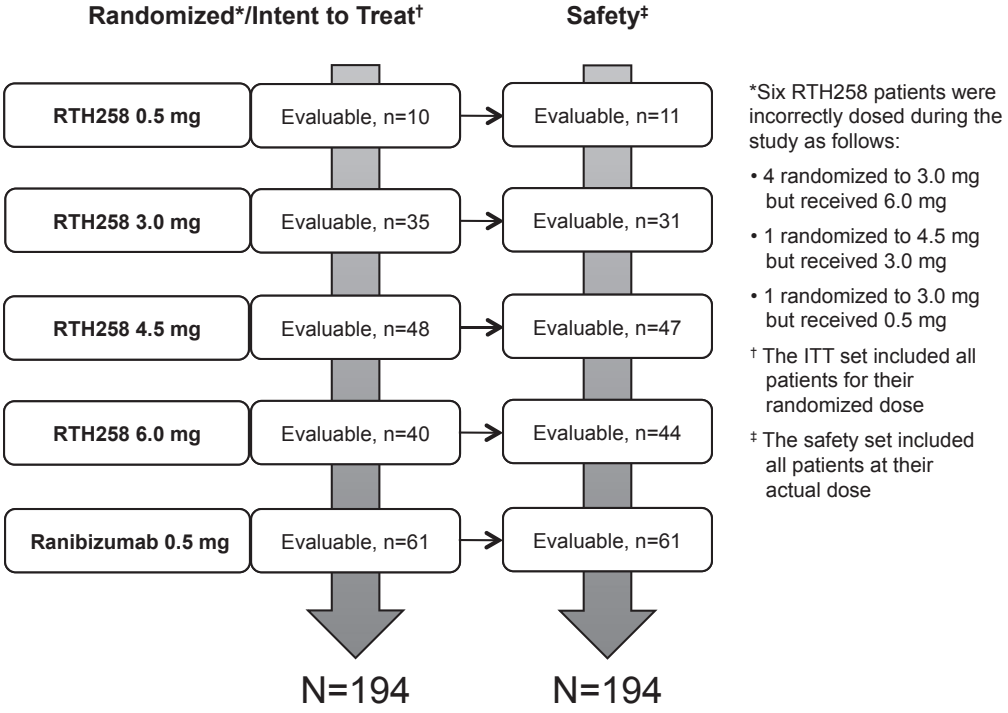


Figure 1. Patient disposition. ITT = intent-to-treat.

Table 3. Patient Demographics and Baseline Characteristics (Safety-Analysis Set)

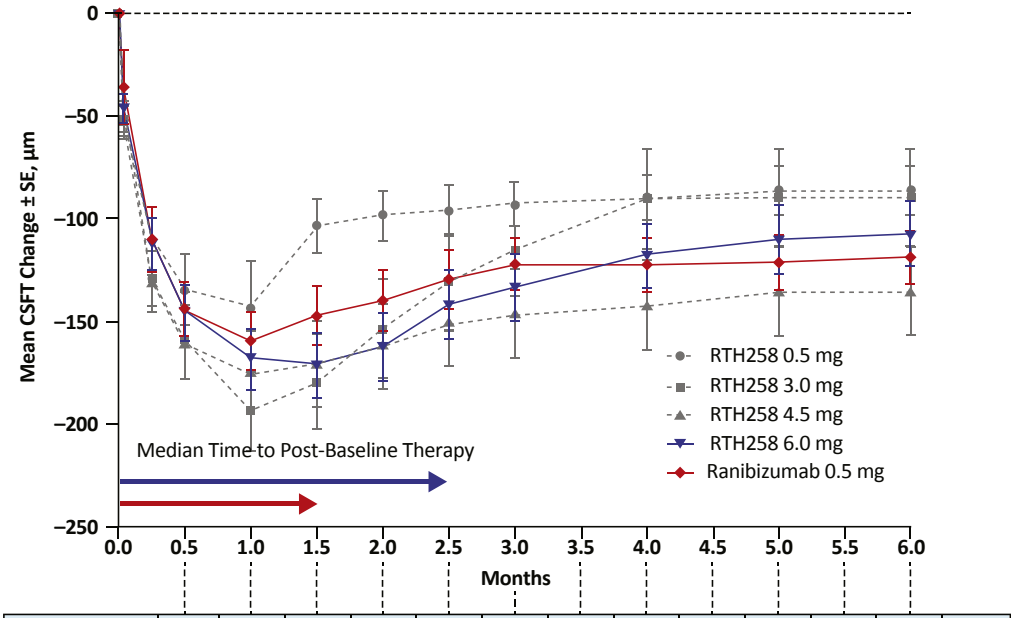
	RTH258 0.5 mg (n = 11)	RTH258 3.0 mg (n = 31)	RTH258 4.5 mg (n = 47)	RTH258 6.0 mg (n = 44)	Ranibizumab 0.5 mg (n = 61)	Overall (N = 194)
Mean (SD) age	75.7 (6.5)	78.4 (8.3)	75.3 (7.7)	74.8 (9.8)	77.8 (8.1)	76.5 (8.4)
Sex, n (%)						
Male	5 (45.5)	19 (61.3)	21 (44.7)	15 (34.1)	28 (45.9)	88 (45.4)
Female	6 (54.5)	12 (38.7)	26 (55.3)	29 (65.9)	33 (54.1)	106 (54.6)
Race, n (%)						
White	11 (100.0)	31 (100.0)	47 (100.0)	43 (97.7)	61 (100.0)	193 (99.5)
American Indian or Alaska Native	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.3)	0 (0.0)	1 (0.5)
Mean (SD) days since diagnosis	29.6 (20.6)	18.9 (15.7)	24.1 (32.3)	21.0 (14.8)	24.0 (19.9)	22.8 (22.1)
Mean (SD) BCVA letters	63.6 (8.6)	57.9 (12.6)	56.8 (10.8)	54.9 (10.8)	56.6 (11.3)	56.9 (11.2)
Mean (SD) CSFT, µm	526.3 (102.9)	530.3 (177.6)	534.6 (166.1)	498.1 (115.8)	509.5 (121.9)	517.3 (140.9)

BCVA = best-corrected visual acuity; CSFT = central subfield thickness; SD = standard deviation.

inclusion criteria), a vision loss of ≥7 letters of BCVA associated with an accumulation of retinal fluid compared with the previous visit, or a new intraocular hemorrhage deemed clinically significant. After day 14 and month 1, the need for PBT was evaluated at 1.5, 2.0, 2.5, 3, 4, 5, and 6 months using the listed criteria. The choice of PBT was at the discretion of the investigator on a patient-by-patient basis in accord with the respective approved product information, as was therapy for the nonstudy eye throughout the study.

RTH258 serum concentration at each collection time point was measured using a validated enzyme-linked immunosorbent assay method. These data were analyzed using a noncompartmental pharmacokinetic method to calculate maximum concentration, area under the curve (AUC) from time 0 to last measurable time point, AUC from time 0 extrapolated to infinity, and half-life for each patient.

The primary safety evaluation in this study was the incidence of AEs occurring in the study eye within 7 days of the intravitreal injection. In addition, anti-RTH258 serum antibodies were determined, and the following safety parameters were assessed: ocular signs, posterior segment, abnormalities, evaluation of retinal hemorrhage/detachment, evaluation of vitreous hemorrhage



RTH258 0.5 mg	0.0	9.1	36.4	72.7	81.8	90.9	---	90.9	---	90.9	---	100.0	*n=11*
RTH258 3.0 mg	6.5	6.5	16.1	29.0	48.4	61.3	---	67.7	---	83.9	---	93.5	*n=31*
RTH258 4.5 mg	0.0	8.5	27.7	36.2	51.1	70.2	---	72.3	---	78.7	---	85.1	*n=47*
RTH258 6.0 mg	2.3	9.1	29.5	34.1	45.5	59.1	---	65.9	---	75.0	---	84.1	*n=44*
Ranibizumab 0.5 mg	3.3	4.9	27.9	57.4	70.5	75.4	---	83.6	---	85.2	---	88.5	*n=61*

Percentage of LVCF data by time point

Figure 2. Central subfield thickness (CSFT) mean change from baseline to month 6; safety-analysis set. Data were censored after administration of post-baseline therapy (PBT) and reflect the last value carried forward for each patient after administration of study drug. The percentage of last value carried forward (LVCF) patient data are shown in the chart under the graph.

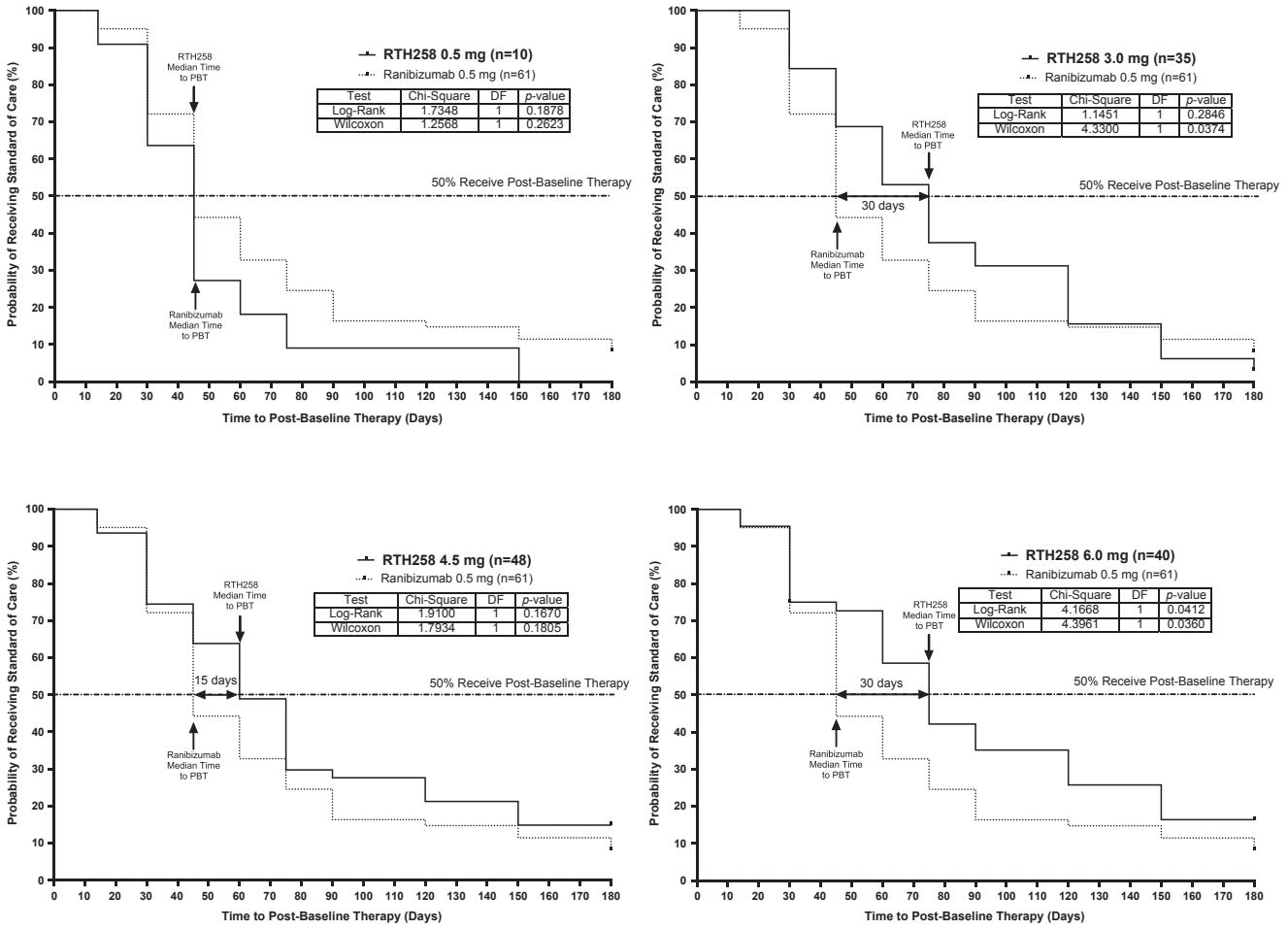


Figure 3. Kaplan–Meier curves; time to receiving post-baseline therapy (PBT); safety-analysis set. DF = degrees of freedom.

density and vitreous cells, intraocular pressure, eyelid/pupil responsiveness, postinjection evaluation of the study eye, physical examination, vital signs, clinical laboratory tests, and all other AEs.

Statistical Analyses

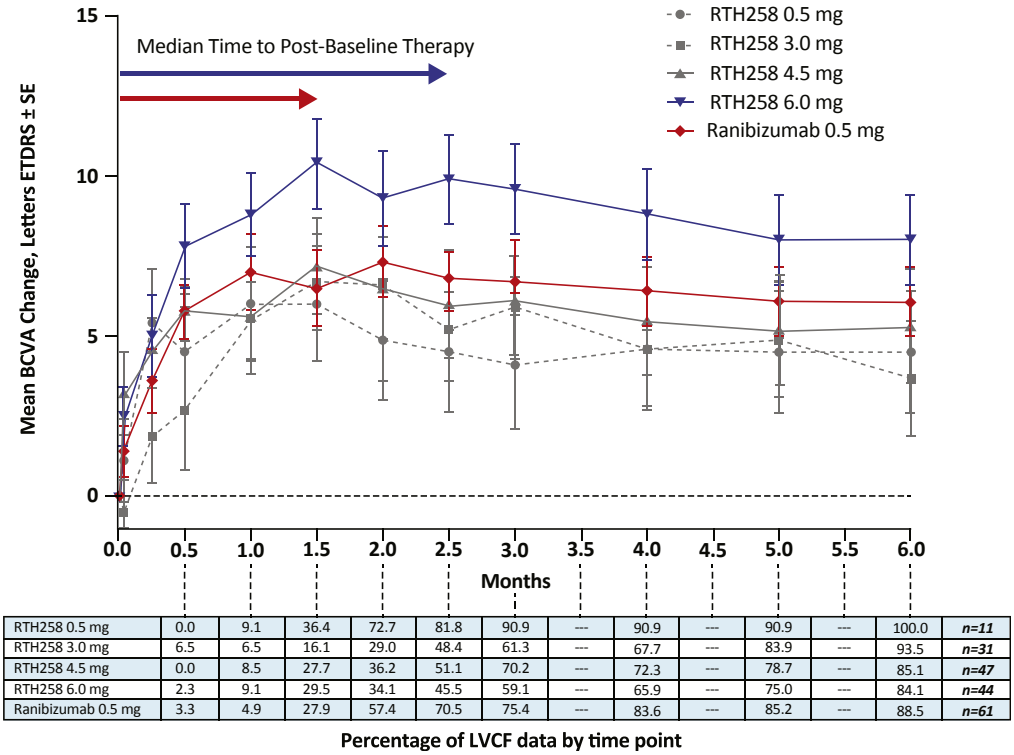
The efficacy analysis was initially to be conducted using the intent-to-treat (ITT) patients. The ITT set included all patients who were randomized, received study drug, and completed ≥1 scheduled on-therapy study visit. The safety set included all patients who received study drug with treatment group allocation according to the actual treatment received. Because 6 patients receiving RTH258 did not receive the correct treatment doses when randomized on day 0, an ad hoc efficacy analysis was performed on the basis of actual treatment received (i.e., the safety analysis set). There was no indication that the misdosed patients in the RTH258 treatment arms constituted a source of a systematic bias. For this phase 1/2 proof-of-concept study, an as-treated efficacy analysis was conducted to allow for the most relevant evaluation of efficacy. Sensitivity analysis of efficacy comparing the outcome of the analysis based on the ITT set and safety set led to the same conclusions. Other amendments to the study protocol are reported in the Appendix (available at www.aaojournal.org).

To eliminate a potential positive effect of PBT on the time-course of CSFT and BCVA, the data presented are based on censoring of assessments after receipt of PBT and using the last-observation-carried-forward method to impute censored and missing data. Data after administration of PBT were censored and artificially rendered "missing" to eliminate any effects of PBT. We will refer to this methodology as the last value (on study treatment) carried forward.

Noninferiority hypothesis testing relating the primary end point change from baseline to month 1 in CSFT was prespecified for RTH258 4.5 and 6.0 mg in comparison with ranibizumab. Two-sided 90% confidence intervals (CIs) for the treatment differences were used to assess these hypotheses, with noninferiority being concluded if the lower limit was greater than −40 μm (the noninferiority margin, derived from historical data). Corresponding CIs were derived from pairwise analysis of variance models with treatment and baseline CSFT category (<400, 400–600, and >600 μm) as factors in the model. No adjustment was planned for multiple testing. Details regarding the determination of sample sizes for the study are available in the Appendix (available at www.aaojournal.org).

The Kaplan–Meier method was used to assess the distribution of time from the initial study drug injection to the receipt of PBT. Treatment differences regarding the supportive end point BCVA were assessed using 90% CIs derived from an analysis of variance model with treatment and baseline BCVA category (<55 and ≥55 letters) as factors.

The independent safety review at each dose escalation approved ascension to the next higher dose up to the MFD and preserved



RTH258 0.5 mg	0.0	9.1	36.4	72.7	81.8	90.9	---	90.9	---	90.9	---	100.0	*n=11*
RTH258 3.0 mg	6.5	6.5	16.1	29.0	48.4	61.3	---	67.7	---	83.9	---	93.5	*n=31*
RTH258 4.5 mg	0.0	8.5	27.7	36.2	51.1	70.2	---	72.3	---	78.7	---	85.1	*n=47*
RTH258 6.0 mg	2.3	9.1	29.5	34.1	45.5	59.1	---	65.9	---	75.0	---	84.1	*n=44*
Ranibizumab 0.5 mg	3.3	4.9	27.9	57.4	70.5	75.4	---	83.6	---	85.2	---	88.5	*n=61*

Percentage of LVCF data by time point

Figure 4. Best-corrected visual acuity (BCVA) mean change from baseline to month 6; safety-analysis set. Data were censored after administration of post-baseline therapy (PBT) and reflect the last value carried forward for each patient after administration of study drug. The percentage of last value carried forward patient data are shown in the chart under the graph. ETDRS = Early Treatment Diabetic Retinopathy Study.

masking for the study investigators and study coordinators for all patients during the dose escalation phase of the study. Therefore, patients from both phases of the study were pooled together for the efficacy and safety assessments.

Results

Patients

In total, 194 patients were randomized and received intravitreal injections of the study drug. Figure 1 shows the patient disposition in the study. All patients randomized to the RTH258 0.5- and 3.0-mg doses completed the study, and 1 patient each in the RTH258 4.5-mg, RTH258 6.0-mg, and ranibizumab 0.5-mg groups discontinued (each because of an AE not related to study treatment) before study completion. All 194 patients were included in the ITT and safety analysis sets. During the study, 6 patients receiving RTH258 were incorrectly dosed: Four patients randomized to receive 3.0 mg received 6.0 mg, 1 patient randomized to receive 4.5 mg received 3.0 mg, and 1 patient randomized to receive 3.0 mg received 0.5 mg.

The majority of the 194 patients were white (99.5%) and aged ≥65 years (91.2%), with a mean (standard deviation [SD]) age of 76.5 (8.4) years. For all patients, the mean (SD) time from the diagnosis of neovascular AMD in the study eye to randomization was 22.8 (22.1) days, and the BCVA in letters read at baseline was 56.9 (11.2). The mean (SD) CSFT at baseline was 517.3 (140.9) μm. Of the 194 patients, 156 (80.4%) had a unilateral diagnosis at baseline. The patient demographic characteristics in the safety set are summarized in Table 3; there were no clinically meaningful differences between the treatment groups.

Efficacy

For the as-treated patients in the safety-analysis set, the least squares mean difference in the mean change from baseline to month 1 in CSFT revealed a 21.09 (90% CI, −8.83 to 51.00) μm larger reduction for the RTH258 4.5-mg group compared with the ranibizumab 0.5-mg group. The least squares mean difference in the mean change from baseline to month 1 in CSFT was 10.34 (90% CI, −18.18 to 38.86) μm in favor of the RTH258 6.0-mg group versus the ranibizumab 0.5-mg group. In both comparisons, the lower bound of the 90% CI was greater than the non-inferiority margin of −40 μm, demonstrating the noninferiority of RTH258 to ranibizumab in the mean change from baseline in CSFT to month 1. The results obtained for the safety-analysis set were consistent with those obtained for the ITT set.

The time course of mean CSFT change from baseline (Fig 2) showed a numeric advantage for the RTH258 3.0-, 4.5-, and 6.0-mg groups when compared with the ranibizumab 0.5-mg group from day 7 to month 2.5. The 3.0-, 4.5-, and 6.0-mg doses of RTH258 had similar profiles up to month 1, after which there was a tendency for CSFT to increase in the RTH258 3.0- and 4.5-mg groups, and the ranibizumab 0.5-mg group. In contrast, CSFT observed in the RTH258 6.0-mg group remained stable between month 1 and month 1.5, with an increase occurring toward month 2. In total, 9 patients had received PBT by day 14, 1 patient in the 0.5-mg RTH258 group, 3 patients in the 4.5-mg RTH258 group, 2 patients in the 6.0-mg group, and 3 patients in the ranibizumab group.

On the basis of the time to PBT, a trend toward a longer duration of effect was observed in each RTH258 group relative to the ranibizumab 0.5-mg group, except in the RTH258 0.5-mg group. At month 1, 4 of 31 patients in the RTH258 3.0-mg

Table 4. Descriptive Statistics for Pharmacokinetic Parameters by Treatment

Pharmacokinetic Parameter	n	Mean (SD)	Harmonic Mean (SD)
Overall			
t_{last}, d	110	12.7 (5.36)	
$t_{1/2}$, d	68	6.2 (3.74)	5.01 (2.97)
RTH258, mg			
0.5			
C_{max}, ng/ml	9	1.50 (0.85)	
t_{last}, d	4	9.3 (3.20)	
$AUC_{0\text{-}last}$, ng*d/ml	4	12.19 (4.86)	
$AUC_{0\text{-}inf}$, ng*d/ml	1	25.9 (—)*	
$t_{1/2}$, d	1	11.0 (—)*	11.0 (—)*
3.0			
C_{max}, ng/ml	30	5.61 (6.22)	
t_{last}, d	24	11.0 (3.43)	
$AUC_{0\text{-}last}$, ng*d/ml	24	34.33 (28.74)	
$AUC_{0\text{-}inf}$, ng*d/ml	13	63.4 (33.6)	
$t_{1/2}$, d	13	7.1 (6.24)	4.86 (4.30)
4.5			
C_{max}, ng/ml	45	5.71 (2.59)	
t_{last}, d	41	12.6 (4.76)	
$AUC_{0\text{-}last}$, ng*d/ml	41	41.46 (20.29)	
$AUC_{0\text{-}inf}$, ng*d/ml	26	62.7 (24.7)	
$t_{1/2}$, d	26	5.7 (2.70)	4.89 (2.28)
6.0			
C_{max}, ng/ml	43	8.53 (7.27)	
t_{last}, d	41	14.3 (6.51)	
$AUC_{0\text{-}last}$, ng*d/ml	41	64.18 (51.41)	
$AUC_{0\text{-}inf}$, ng*d/ml	28	83.2 (52.6)	
$t_{1/2}$, d	28	6.2 (3.01)	5.10 (2.78)

$AUC_{0\text{-}inf}$ = area under the curve from time 0 extrapolated to infinity; $AUC_{0\text{-}last}$ = area under the curve from time 0 to last measurable time point; C_{max} = maximum concentration; SD = standard deviation; $t_{1/2}$ = half-life; t_{last} = time to last measureable time point.
*SD not calculable because n = 1.

group, 12 of 47 patients in the RTH258 4.5-mg group, 11 of 44 patients in the RTH258 6.0-mg group, and 17 of 61 patients in the ranibizumab 0.5-mg group had received PBT. On the basis of the Kaplan–Meier analysis, the median times to receiving PBT for patients in the RTH258 0.5-, 3.0-, 4.5-, and 6.0-mg groups were 45, 75, 60, and 75 days, respectively, whereas the median time was 45 days with ranibizumab. These differences between the RTH258 treatment groups and the ranibizumab 0.5-mg group (Fig 3) were assessed using the Wilcoxon test ($P = 0.26$, 0.04, 0.18, and 0.04 for the 0.5-, 3.0-, 4.5-, and 6.0-mg groups, respectively).

The mean changes from baseline in BCVA observed in the RTH258 6.0-mg group were consistently larger than those observed in the ranibizumab 0.5-mg group at all post-baseline study visits (Fig 4). The mean and the corresponding 90% CI for the BCVA change from baseline suggested potential advantages for RTH258 6.0 mg, specifically at months 1 (−1.99 [90% CI, −4.87 to 0.89] letters), 1.5 (−4.05 [90% CI, −7.10 to −1.00] letters), and 2 (−2.08 [90% CI, −5.13 to 0.96] letters).

The time course of mean BCVA change from baseline (Fig 4) observed in the RTH258 0.5-, 3.0-, and 4.5-mg groups was generally consistent with the time course observed in the ranibizumab 0.5-mg group. Although the BCVA tended to worsen in the ranibizumab 0.5-mg group after month 1, it further improved between months 1 and 1.5 in the RTH258 3.0-, 4.5-, and 6.0-mg groups.

Pharmacokinetics of RTH258

Serum RTH258 levels were low but quantifiable in most patients after a single intravitreal injection of RTH258. The maximum concentration and AUC values increased with each dose, and the peak serum RTH258 concentration was observed in the first time point after dosing (1 day); after that, the RTH258 concentration demonstrated a mono-exponential decay, with an overall harmonic mean (SD) half-life of 5.10 (2.78) days. Pharmacokinetic parameters by each RTH258 dose are summarized in Table 4.

Safety

The safety population comprised 194 patients who were exposed to the study drug. Treatment-emergent AEs are summarized in Table 5. The most frequent AEs related to the administration procedure reported among patients treated with RTH258 were conjunctival hemorrhage, eye pain, and conjunctival hyperemia; the majority of these events were mild in intensity and resolved within a few days without the need for treatment. The features of these events were not uncharacteristic of those observed for intravitreal injections in general, and no cases of endophthalmitis were reported. No patient exposed to RTH258 discontinued study participation because of a treatment-emergent AE. Seventeen patients experienced serious AEs: 7 of 61 patients in the ranibizumab and 4 of 31, 3 of 47, and 3 of 44 patients in the RTH258 3.0-mg, 4.5-mg, and 6.0-mg groups, respectively. None of the reported serious AEs were considered related to treatment.

In Table 5, hypertension AEs are reported only in patients who were randomized to RTH258. These AEs were all reported on a change in concomitant medication and not related to measured blood pressure at scheduled or unscheduled visits. In 6 of the 8 patients, the change in concomitant medication occurred 40 to 140 days post-RTH258 administration and after receipt of PBT in 3 of these 6 patients. Three similar instances of concomitant blood pressure–lowering medication changes in hypertensive patients randomized to ranibizumab were reported as disease change rather than AE, and a single instance of an AE "blood pressure systolic increased" in a fourth patient randomized to ranibizumab is not included in Table 5.

Discussion

In this phase 1/2 proof-of-concept study, RTH258 met the primary objective of demonstrating noninferiority (margin: 40 μm, 1-sided alpha 0.05) to ranibizumab 0.5 mg in the change in CSFT from baseline to month 1 after a single intravitreal injection of the RTH258 4.5- or 6.0-mg dose. The mean change in CSFT over time showed a numerically better outcome for RTH258 3.0, 4.5, and 6.0 mg when compared with ranibizumab 0.5 mg from day 7 to month 2.5. After a single intravitreal injection, RTH258 3.0, 4.5, and 6.0 mg led to a greater median time to receiving PBT (75, 60, and 75 days, respectively) when compared with ranibizumab 0.5 mg (45 days), suggesting a more durable response for the majority of patients. Likewise, the time course of the change in BCVA showed improvement between months 1 and 1.5 with RTH258 at 3.0, 4.5, and 6.0 mg, whereas the change in BCVA tended to worsen in the ranibizumab group after month 1. The censoring of assessments after receipt of PBT and using the last value carried

Table 5. Summary of Treatment-Emergent Adverse Events (Safety-Analysis Set)

Patients with AE, n (%)	RTH258 0.5 mg (n = 11)	RTH258 3.0 mg (n = 31)	RTH258 4.5 mg (n = 47)	RTH258 6.0 mg (n = 44)	Ranibizumab 0.5 mg (n = 61)
Serious AEs	0 (0.0)	4 (12.9)	3 (6.4)	3 (6.8)	7 (11.5)
Treatment-related	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Related to injection procedure	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.3)	0 (0.0)
Not related to treatment	0 (0.0)	4 (12.9)	3 (6.4)	2 (4.5)	7 (11.5)
Most frequent treatment-emergent AEs; occurring in ≥3 patients in any treatment group					
Conjunctival hemorrhage	0 (0.0)	3 (9.7)	3 (6.4)	8 (18.2)	2 (3.3)
Nasopharyngitis	1 (9.1)	0 (0.0)	3 (6.4)	3 (6.8)	2 (3.3)
Retinal hemorrhage	0 (0.0)	1 (3.2)	2 (4.3)	1 (2.3)	4 (6.6)
Visual acuity reduced	0 (0.0)	2 (6.5)	1 (2.1)	2 (4.5)	3 (4.9)
Hypertension	0 (0.0)	1 (3.2)	5 (10.6)	2 (4.5)	0 (0.0)
Urinary tract infection	0 (0.0)	4 (12.9)	1 (2.1)	2 (4.5)	0 (0.0)
Eye pain	0 (0.0)	0 (0.0)	4 (8.5)	1 (2.3)	1 (1.6)
Bronchitis	0 (0.0)	0 (0.0)	3 (6.4)	0 (0.0)	3 (4.9)
Pneumonia	0 (0.0)	1 (3.2)	0 (0.0)	0 (0.0)	3 (4.9)
Back pain	0 (0.0)	0 (0.0)	3 (6.4)	0 (0.0)	1 (1.6)
Patients with ≥1 treatment-emergent AE related to study drug					
Iritis	0 (0.0)	0 (0.0)	1 (2.1)	1 (2.3)	0 (0.0)
Iridocyclitis	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.6)
Maculopathy	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.6)
Vitritis	0 (0.0)	0 (0.0)	1 (2.1)	0 (0.0)	0 (0.0)
Patients with ≥1 treatment-emergent AE related to the administration procedure					
Conjunctival hemorrhage	0 (0.0)	3 (9.7)	2 (4.3)	6 (13.6)	1 (1.6)
Eye pain	0 (0.0)	0 (0.0)	2 (4.3)	1 (2.3)	0 (0.0)
Conjunctival hyperemia	0 (0.0)	1 (3.2)	0 (0.0)	0 (0.0)	1 (1.6)
Choroiditis	0 (0.0)	0 (0.0)	1 (2.1)	0 (0.0)	0 (0.0)
Corneal disorder	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.6)
Dry eye	0 (0.0)	1 (3.2)	0 (0.0)	0 (0.0)	0 (0.0)
Eye irritation	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.6)
Foreign body sensation in eyes	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.3)	0 (0.0)
Visual acuity reduced	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.3)	0 (0.0)
Vitreous floaters	0 (0.0)	0 (0.0)	1 (2.1)	0 (0.0)	0 (0.0)

AE = adverse event.

forward method to impute censored and missing data were designed to eliminate a potential positive effect of PBT on the time course of CSFT and BCVA.

The safety profile of RTH258 in this single-dose study was consistent with the known safety profile of intravitreally injected anti-VEGF agents and did not show any unexpected findings. No patient in the RTH258 dose groups discontinued the study because of a treatment-emergent AE, and there were no reports of endophthalmitis. In the phase 2 OSPREY study, 6 mg of RTH258 was dosed over 1 year and well tolerated.[17]

Visual acuity (BCVA) remains an important primary outcome assessment in clinical trials of treatments for neovascular AMD, and at 1 year the outcome was the primary end point in the Anti-VEGF Antibody for the Treatment of Predominantly Classic Choroidal Neovascularization in AMD (ANCHOR),[9] Minimally Classic/Occult Trial of the Anti-VEGF Antibody Ranibizumab in the Treatment of Neovascular AMD (MARINA),[10] and VEGF Trap-Eye: Investigation of Efficacy and Safety in Wet AMD 1 and 2 (VIEW 1 and 2)[18] pivotal trials, validating visual acuity as a viable long-term study assessment.[19,20] In addition, current SD OCT systems provide greatly improved images of the intraretinal cysts, intraretinal fluid, subretinal fluid, and subretinal pigment epithelium fluid compared with time-domain optical coherence tomography.[21] In this proof-of-concept study, with a primary end point at month 1, treatment consisting of a single intravitreal injection, and small sample sizes over multiple dose groups, CSFT was chosen as a noninvasive and objective assessment[21] of anatomic response to treatment that is more sensitive than BCVA at the 1-month time point. The EXCITE study compared monthly with quarterly ranibizumab treatment regimens over the course of 1 year using both BCVA and retinal morphology assessed by optical coherence tomography, which demonstrated a stronger effect size for optical coherence tomography compared with BCVA at 1 month.[22] Although improvement in visual acuity was noted in this study, longerterm, multidose studies will be needed to objectively compare RTH258 with other anti-VEGF treatments in terms of changes in BCVA. One such trial (NCT01796964) currently is being analyzed.

Compared with ranibizumab 0.5 mg, the median time to PBT for the RTH258 3.0-, 4.5-, and 6.0-mg doses were observed to be longer by approximately 15 to 30 days. As a surrogate estimate of durability, this result suggests that the smaller (26 kDa) RTH258, at a higher concentration of active drug (6.0 mg; i.e., a 22-fold molar amount) with a high binding affinity for VEGF with a KD of 104 pM compared with a KD of 96 pM for aflibercept, <171 pM for

ranibizumab, and 3300 pM for bevacizumab,[15,16] may have accounted for an increase in the durability of the response to treatment. Results from this study also indicate the safety and tolerability of the high RTH258 6.0-mg dose, suggesting that it has potential to show clinical efficacy at dosing intervals longer than 1 month.

Study Limitations

A limitation of the study relates to the varied volumes administered for each of the different RTH258 study doses. Although a relatively small overall study population, the sample size was adequate to assess noninferiority of RTH258 versus ranibizumab on the basis of an anatomic response to treatment 1 month after injection. Another limitation concerns the revised efficacy analysis using the as-treated patients (safety set) rather than the ITT patient set as originally planned in the study protocol. However, sensitivity analysis of efficacy comparing the outcome of the analysis based on the ITT set and safety set led to the same conclusions.

In conclusion, this first-in-human, proof-of-concept study of RTH258 compared with ranibizumab for the treatment of wet AMD demonstrated noninferiority (margin: 40 μm, 1-sided alpha 0.05) in the 1-month change in CSFT at 4.5- and 6.0-mg doses, with an observed increase of 30 days in the median time to administering PBT for the 6.0-mg dose. There were no unexpected safety concerns with RTH258 at any dose level, and the results support the continued development of RTH258 at the 6.0-mg dose for the treatment of wet AMD. Full results of the phase 2 study OSPREY[17] will be reported soon, and phase 3 studies have started enrolling patients (NCT02307682 and NCT02434328).

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Abbreviations and Acronyms:
AE = adverse event; **AMD** = age-related macular degeneration; **AUC** = area under the curve; **BCVA** = best-corrected visual acuity; **CI** = confidence interval; **CSFT** = central subfield thickness; **ITT** = intent-to-treat; **MFD** = maximum feasible dose; **PBT** = post-baseline therapy; **SD** = standard deviation; **SD OCT** = spectral-domain optical coherence tomography; **VEGF** = vascular endothelial growth factor.

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