Clinical Trials

Dose-Ranging Evaluation of Intravitreal siRNA PF-04523655 for Diabetic Macular Edema (the DEGAS Study)

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Purpose. To evaluate the safety and efficacy of three doses of PF-04523655, a 19-nucleotide methylated double stranded siRNA targeting the RTP801 gene, for the treatment of diabetic macular edema (DME) compared to focal/grid laser photocoagulation.

Methods. This multicenter, prospective, masked, randomized, active-controlled, phase 2 interventional clinical trial enrolled 184 DME patients with best corrected visual acuity (BCVA) of 20/40 to 20/320 inclusive in the study eye. Patients were randomly assigned to 0.4-mg, 1-mg, 3-mg PF-04523655 intravitreal injections or laser. The main outcome measure was the change in BCVA from baseline to month 12.

Results. All doses of PF-04523655 improved BCVA from baseline through month 12. At month 12, the PF-04523655 3-mg group showed a trend for greater improvement in BCVA from baseline than laser (respectively 5.77 vs. 2.39 letters; $P = 0.08$; 2-sided $\alpha = 0.10$). The study was terminated early at month 12 based on predetermined futility criteria for efficacy and discontinuation rates. PF-04523655 was generally safe and well-tolerated, with few adverse events considered treatment-related. By month 12, the discontinuation rates in the PF-04523655 groups were higher than the laser group and were inversely related to dose levels.

Conclusions. PF-04523655 showed a dose-related tendency for improvement in BCVA in DME patients. Studies of higher doses are planned to determine the optimal efficacious dose of PF-04523655. PF-04523655 may offer a new mode of therapeutic action in the management of DME. (ClinicalTrials.gov number, NCT00701181.) (*Invest Ophthalmol Vis Sci.* 2012;53:7666–7674) DOI:10.1167/iovs.12-9961

Diabetic retinopathy (DR) remains the major threat to sight in the working age population in the developed world. DR affects over 5.3 million people in the United States, making up to 8% of all cases of legal blindness and 12% of newly diagnosed blindness.[1–3] Although severe vision loss occurs from proliferative diabetic retinopathy, diabetic macular edema (DME) accounts for functional vision loss in the majority of diabetic patients.[4,5] The early treatment diabetic retinopathy study (ETDRS) demonstrated the efficacy of focal/grid photocoagulation in reducing the risk of moderate vision loss from DME.[5] The efficacy of focal/grid laser photocoagulation for the treatment of DME continues to be confirmed and presently is standard of care[6]; however, recent studies have demonstrated that intravitreal injections of anti-VEGFs provide a statistically significant improvement in visual acuity and reduce retinal thickness more than focal/grid laser photocoagulation in DME patients.[7–9] The improved efficacy achieved with anti-VEGFs is consistent with the observation that VEGF increases vascular permeability and VEGF levels are considerably higher in DME patients with extensive macular leakage than in patients with minimal leakage.[10,11]

Ideally, the genetic mechanism that leads to the extracellular secretion of VEGF could be suppressed by interfering with the responsible genes or any of the steps that are required for the production of VEGF. VEGF expression is regulated by hypoxia inducible factor.[12] PF-04523655 is an *O*-methyl stabilized small interfering ribonucleic acid (siRNA) that acts via RNA interference to inhibit expression of a hypoxia-inducible gene, RTP801.[13] Expression of RTP801 has been shown to be upregulated in streptozotocin-induced diabetic mice and rats (Rittenhouse KD, et al. *IOVS* 2011;52:ARVO E-Abstract 5641).[14] Intravitreal PF-04523655 suppresses the expression of RTP801 in the retina of these diabetic models (Rittenhouse KD, et al. *IOVS* 2011;52:ARVO E-Abstract 5641; and Rittenhouse KD, et al. *IOVS* 2010;51: ARVO E-Abstract 6447). The different mechanism of action of PF-04523655 (i.e., blocking the RTP801 hypoxia/stress pathway) may provide a new treatment option, which is independent of, and possibly complementary to, the mechanism of anti-VEGF therapies for the treatment of DME.[7–9,15–18]

In view of the evidence of preclinical safety and efficacy of PF-04523655, a 3-year, phase 2 clinical trial was designed to evaluate three dose levels (0.4, 1, and 3 mg) of PF-04523655 compared with focal/grid laser photocoagulation in patients with visual loss secondary to DME. Since the mechanism of action of PF-04523655 is novel, three interim analyses at 3, 6, and 12 months were preplanned to assess possible early and/or late efficacy/safety signals. In addition, to minimize risk to the patients, patients were rescued and discontinued from the study according to predetermined criteria.

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METHODS

Study Design

This study was a randomized, prospective, multicenter, dose ranging, controlled clinical trial of the safety and efficacy of PF-04523655 versus focal/grid laser photocoagulation for the treatment of DME.

This study was approved by institutional review boards/ethic committees before its execution. All patients read and signed an informed consent in accordance with Good Clinical Practices, the World health Organization Declaration of Helsinki 1996, and Health Insurance Portability and Accountability Act. The safety of the trial was assessed by an independent data monitoring committee (DMC).

The study was originally designed for a 36-month study period with a primary efficacy endpoint measured at month 24. The preplanned interim analyses were performed when approximately 120 patients completed 3, 6, and 12 months.

Study Population

Patients were recruited at 46 retina practices in the United States, Peru, Italy, Israel, India, the United Kingdom, Germany, and Denmark (see DEGAS Clinical Study Group). Consented patients of either sex, $\geq$18 years old with type 1 or 2 diabetes, best-corrected visual acuity (BCVA) of 20/40 to 20/320 inclusive (73 to 24 ETDRS letters inclusive) and time-domain optical coherence tomography (OCT) central subfield retinal thickness $\geq$275 μm in the study eye due to DME were enrolled. Other major inclusion criteria were BCVA in the fellow eye of $\geq$19 letters, serum HbA1c $\geq$5.5% and $\leq$12%, and treatment for DME with laser photocoagulation could be delayed for at least 90 days after enrollment. The key exclusion criteria were pan-retinal photocoagulation or macular photocoagulation performed in the study eye within 3 months of the screening visit, a high risk of developing proliferative diabetic retinopathy in the study eye, or a systemic blood pressure >180/110 mm Hg.

Randomization and Masking

Only one eye was treated (study eye); in the event both eyes were eligible, the study eye was selected by the investigator and patient. Patients were randomly assigned with a 1:1:1:1 allocation ratio to 0.4-mg, 1-mg, 3-mg PF-04523655 or laser. Randomization was performed centrally via an interactive response system and was stratified by screening BCVA (<55 versus $\geq$55 letters). Patients were masked to the different doses of PF-04523655, but not to laser. The investigator administering the study treatments was not masked to the treatment the patient received; however, study personnel who measured and evaluated BCVA, fundus photography (FP), fundus fluorescein angiography (FFA) and OCT (Stratus; Carl Zeiss Meditec, Dublin, CA) were masked throughout the study period to the treatments regardless of whether the treatments were PF-04523655 or laser.

PF-04523655 Treatments

In vitro and in vivo experiments conducted to gain insights into the general kinetics of siRNA demonstrated that gene silencing in nondividing cells can last for approximately 3 weeks.[19] Consistent with these findings, PF-04523655 suppressed the development of neovascularization for a full 3 weeks in a nonhuman primate model of laser-induced choroidal neovascularization (Quark, data on file). Based on these observations and considering patient convenience, PF-04523655 was administered every 4 weeks.

In patients randomized to PF-04523655, 100 μL of the study medication was administered once every month via intravitreal (IVT) injection for 6 months in the study eye. After the month 6 visit, PF-04523655 was administered on an as needed (PRN) basis, providing the central subfield retinal thickness was greater than 250 μm and the patient did not have a BCVA or OCT measurement that met rescue criteria (see "Rescue Therapy" below). The investigators could withdraw any patient at their discretion for any reason, including poor glycemic control and/or poor renal function.

Laser Treatments

Patients randomized to laser treatment received laser Modified ETDRS technique) at baseline. Focal/grid photocoagulation with a green to yellow wavelength laser was used to treat all leaking microaneurysms in areas of retinal thickening between 500 and 3500 μm temporally and 500 and 3000 μm inferiorly, superiorly, and nasally from the center of the macular, but not within 500 μm of the optic disc. The laser spot size and duration were 0.50 μm and 0.05 to 0.10 seconds, respectively. Laser burns were separated by two visible burn widths and required to appear light gray to white beneath all treated microaneurysms. Retreatment was assessed every 3 months to determine if laser therapy should be deferred. At the month 3 visit, laser treatment was deferred when either of the following criteria were met: the central subfield retinal thickness was $\leq$250 μm; the central subfield was >250 μm and the excess central subfield thickening (200 μm was used for the reference) had decreased by at least 50% from baseline; or there was a five-letter or more gain in BCVA from baseline. After the month 6 visit, focal/grid laser photocoagulation was deferred whenever the central subfield retinal thickness was $\leq$250 μm.

Rescue Therapy

After the month 3 visit, patients receiving PF-04523655 could be rescued with laser, if there was a >10 letter loss in BCVA or an increase in central subfield retinal thickness >100 μm from baseline. In order to evaluate the true treatment effect from PF-04523655, patients in the PF-04523655 who required rescue therapy were discontinued from the study at the time of rescue and their last BCVA/OCT/FFA measurements taken before discontinuation were carried forward for the primary and secondary efficacy analyses (last observation carried forward; LOCF). Patients in the laser group could also be discontinued to receive available rescue therapy at the discretion of the investigators.

Nonstudy Treatments

During the study, patients were permitted to receive laser therapy, anti-VEGF therapy, or glucocorticoids in the nonstudy eye, but were not permitted to receive systemic glucocorticoids.

Outcome Measures and Follow-up

The original primary outcome measure was the mean change in BCVA from baseline at the month 24 visit; however, this was changed to month 12 since the study was terminated early. Key secondary outcome measures included incidence and severity of ocular and systemic adverse events, percent of patients with >10- and >15-letter improvement in BCVA, the proportion of patients with >15-letter deterioration in BCVA, mean change in central subfield retinal thickness, area of fluorescein leakage from baseline, and the mean change in patient self-reported visual functioning and vision-related quality of life as measured by NEI-VFQ-25 composite and 12 subscale scores from baseline. For BCVA and central subfield retinal thickness, missing values were imputed using the LOCF method. No imputation was done for all other secondary endpoints.

Clinical Procedures

BCVA using the EDTRS protocol, intraocular pressure, biomicroscopy, and fundoscopy were accessed at screening, baseline, weeks 1 and 2, and then monthly for the duration of the study. Using precertified equipment, a study-certified photographer or technician performed all FP, FFA, and OCT. All images were assessed by a central reading center (University of Wisconsin, Madison, WI).

Table 1. Demographic and Baseline Characteristics of Patients in the DEGAS Study

	PF-04523655				
	0.4 mg	1 mg	3 mg	Laser Photocoagulation	Total
	N = 46	*N* = 46	*N* = 46	*N* = 46	*N* = 184
Number of patients (%)					
Male	26 (56.5)	27 (58.7)	27 (58.7)	33 (71.7)	113 (61.4)
Female	20 (43.5)	19 (41.3)	19 (41.3)	13 (28.3)	71 (38.6)
Age, y					
Mean (SD)	61.4 (10.4)	60.8 (9.8)	60.7 (11.4)	64.5 (9.9)	61.8 (10.4)
Range	35 to 89	37 to 86	28 to 84	39 to 90	28 to 90
Race, *n* (%)					
White	34 (73.9)	34 (73.9)	38 (82.6)	38 (82.6)	144 (78.3)
Black	4 (8.7)	4 (8.7)	3 (6.5)	2 (4.3)	13 (7.1)
Asian	5 (10.9)	5 (10.9)	4 (8.7)	2 (4.3)	16 (8.7)
Japanese American	1 (2.2)	0	0	0	1 (0.5)
Korean	1 (2.2)	0	0	0	1 (0.5)
Other	3 (6.5)	5 (10.9)	4 (8.7)	2 (4.3)	14 (7.6)
Other	3 (6.5)	3 (6.5)	1 (2.2)	4 (8.7)	11 (6.0)
Primary diagnosis, *n* (%)					
Diabetic retinal edema	46 (100)	46 (100)	46 (100)	46 (100)	184 (100)
Duration since diagnosis, months					
Mean (SD)	32.8 (39.7)	30.0 (56.2)	35.2 (49.8)	29.6 (35.5)	31.9 (45.7)
Range	0.7 to 174.1	0.6 to 357.9	0.9 to 269.5	0.8 to 138.5	0.6 to 357.9
Study eye, *n* (%)					
Left eye	24 (52.2)	17 (37.0)	18 (39.1)	22 (47.8)	81 (44.0)
Right eye	22 (47.8)	29 (63.0)	28 (60.9)	24 (52.2)	103 (56.0)
BCVA of study eye, *n* (%)					
<55	15 (32.6)	19 (41.3)	18 (39.1)	17 (37.0)	69 (37.5)
≥55	31 (67.4)	27 (58.7)	28 (60.9)	29 (63.0)	115 (62.5)
Baseline BCVA, letters					
Mean (SD)	57.4 (12.21)	56.8 (12.03)	56.6 (10.80)	57.7 (12.26)	57.1 (11.75)
Range	27 to 80	21 to 75	29 to 78	18 to 75	18 to 80

Statistical Analysis

The patient population used for the efficacy and safety analyses was the intent-to-treat (ITT) population, which included all randomized patients who received at least one study treatment. Since this was not a confirmatory study, for the sample size estimation, the sponsor considered statistical significance at a 1-sided 0.05 level (2-sided *P* value = 0.10). *P* values were not adjusted for multiple comparisons or interim analyses. By choosing the significance level at a 2-sided *P* value = 0.10 rather than the conventional 2-sided *P* value = 0.05, the sponsor was willing to accept a higher type I error (false positive) rate for the purpose of internal decision making.

For the primary efficacy and numeric secondary efficacy parameters, the difference in mean BCVA change from baseline between any of the PF-04523655 dose groups and the laser group was analyzed using an ANOVA model with treatment group and screening BCVA category as factors.

A sample size of 160 patients (40 patients in each group) was estimated to provide a 78% power at a 2-sided 0.10 significance level to detect a 7-letter difference in mean changes of BCVA between any of the PF-04523655 dose group and the laser group, assuming an SD of 11.8 letters and a dropout rate of 15%.

Results

The ITT population for this study was comprised of 184 patients (*n* = 46 in each group) enrolled at 46 clinical sites. The first patient and first visit was on June 23, 2008, and the last patient's last visit was January 28, 2011. The baseline demographic characteristics (Table 1) and baseline grade of diabetic retinopathy (ETDRS grading[20]); percentage of patients who received panretinal and/or focal/grid laser photocoagulation; OCT central retinal thickness; blood pressure; blood glucose; HbA1c; blood urea nitrogen; serum creatinine; and number of patients with overt nephropathy (Table 2) were comparable among the four treatment groups. The flow of patients through the study is illustrated in Figure 1.

Following the 12-month interim analysis, the study was terminated early based upon internal predetermined (discontinuation rates and change in BCVA from baseline) futility criteria. At months 3, 6, and 12, the discontinuation rates were 13%, 4%, 7%, 7%; 33%, 28%, 20%, 7%; and 57%, 41%, 35%, 15% for the PF-04523655 0.4-mg, 1-mg, 3-mg, and laser groups, respectively. At month 3, the main reasons for patient discontinuation were adverse events and patient withdrawal of consents. At months 6 and 12, the primary reason for patient discontinuation was lack of efficacy. By month 12, a total of 56 patients had been discontinued because of lack of efficacy. These patients had no mean change in BCVA, a mean 30-μm increase in central retinal thickness, and their NEI-VFQ-25 scores were not improved.

Since most patients had not reached the month 24 visit (original primary efficacy endpoint) at the time of study termination, the primary statistical analysis was changed to

Table 2. Baseline Retinal and Laboratory Findings of Patients in the DEGAS Study

	PF-04523655			
	0.4 mg	**1 mg**	**3 mg**	**Laser Photocoagulation**
Retinopathy,[20] ETDRS grade 35 to 53 inclusive	61%	50%	59%	48%
Panretinal and/or focal/grid laser photocoagulation	13%	37%	28%	28%
Central retinal thickness μm, mean (SD)	474 (161)	454 (170)	451 (127)	459 (136.8)
Systolic blood pressure mm Hg, mean (SD)	140 (19)	140 (20)	136 (17)	136 (19)
Diastolic blood pressure mm Hg, mean (SD)	77 (12)	81 (9)	78 (10)	77 (11)
Blood glucose mg/dL, median	105	112	121	131
HbA1c %, median	7.4	7.9	7.7	7.8
Blood urea nitrogen mg/dL, median	38	36	38	41
Serum creatinine mg/dL, median	1.3	1.2	1.3	1.4
Overt nephropathy, number of patients	1	0	2	3

evaluate efficacy at month 12 instead of the prespecified 24-month timepoint.

Efficacy Analysis

The least square (LS) mean BCVA change from baseline at month 12 (LOCF) in the PF-04523655 3-mg group (5.77 letters) showed a trend for greater improvement in BCVA from baseline than the laser group (2.39 letters), with a difference of 3.38 (90% confidence interval [CI]: 0.23–6.53) letters, $P = 0.08$. The differences between the laser group and the PF-04523655 0.4-mg and 1-mg groups were not statistically significant, $P = 0.93$ and $P = 0.35$, respectively (Fig. 2).

At month 12, there was not a statistically significant difference between the PF-04523655 and the laser treatment groups in the proportion of patients who gained ≥10 letters in BVCA; however, there was a trend for a dose response in the PF-04523655 treatment groups for percentage of patients gaining >15 letters more than the laser treatment group. At month 12 (LOCF), the percentage of patients that gained >15 letters for PF-04523655 0.4 mg, 1 mg, and 3 mg were 6.5% (90% CI: −1.2% to 14.2%); 8.7% (90% CI: 0.4% to 17.0%); and 15.2% (90% CI: 5.4% to 25.1%), respectively. However, there was not a meaningful difference between the percentage of patients that lost >15 letters in the PF-04523655 and laser treatment groups, since the confidence intervals of the differences all contained zero. At month 12 (LOCF) for PF-04523655 0.4 mg, 1 mg, and 3 mg, the 90% confidence intervals were: −2.6% to 11.3%, −5.0% to 5.0%, and −3.9% to 8.3%, respectively (Table 3).

In the PF-04523655 dose groups, patients with a screening BCVA <55 letters on average achieved a greater BCVA change from baseline than patients with a screening BCVA ≥55

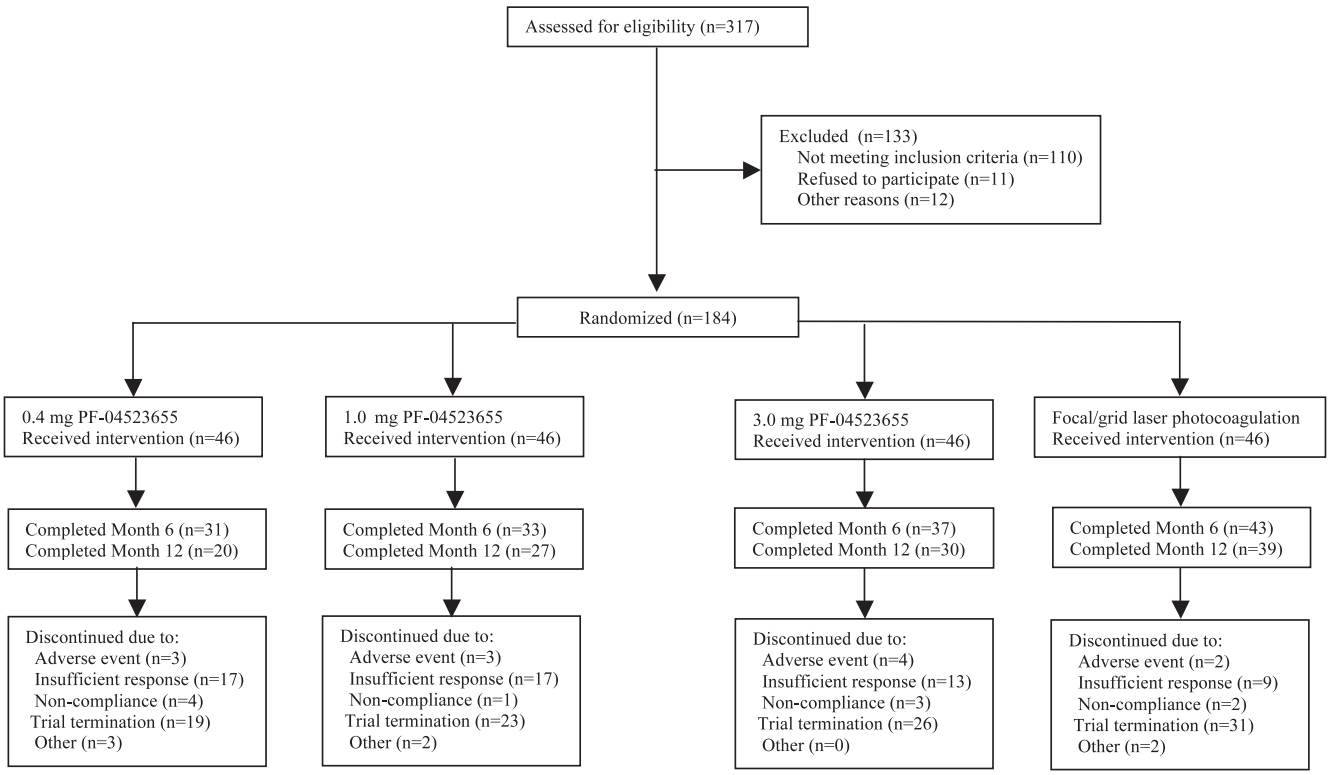

Figure 1. Patient flow in the DEGAS Study.

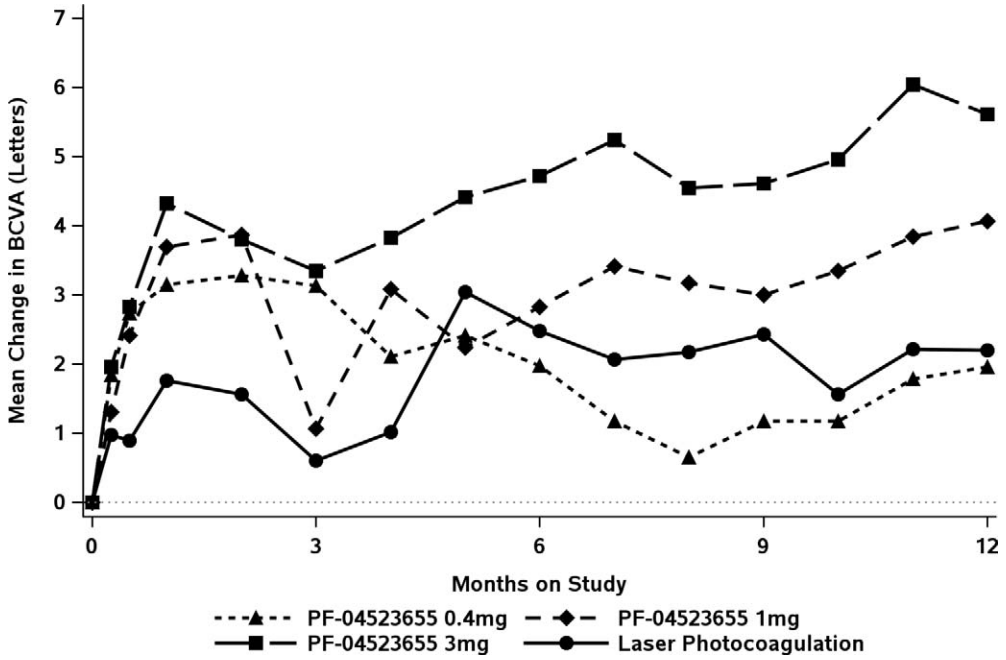

FIGURE 2. Mean change in BCVA (letters) from baseline (LOCF) by study visit, study eye, ITT population.

letters. The reverse was observed in the laser photocoagulation group.

Other Efficacy Measures

Central subfield retinal thickness was reduced in all PF-04523655 dose groups over time, but no dose response was observed. At month 12 (LOCF), the LS mean reduction in central subfield retinal thickness from baseline for the PF-04523655 0.4-mg, 1-mg, 3-mg, and laser groups was 47 μm, 20 μm, 63 μm, and 104 μm, respectively (Fig. 3).

The mean change in total retinal volume and fluorescein leakage from baseline to month 12 showed a gradually decreasing trend in the laser group, while the trends in the PF-04523655 dose groups were unclear and no dose-response pattern was observed (Figs. 4, 5). Compared to baseline, there was no change in the level of diabetic retinopathy in any of the treatment groups.

For the NEI-VFQ-25 Composite Score of patients in the PF-04523655 0.4-mg and 3-mg groups who remained in the study, their scores tended to improve over time, while little changes were seen in patients who remained in the PF-04523655 1-mg and laser groups.

Safety Analysis

Most patients reported a treatment-emergent adverse event (AE), with a slightly lower percentage in the laser group compared with the PF-04523655 groups (91.3%, 93.5%, and 95.7% in the 0.4-mg, 1-mg, and 3-mg PF-04523655 groups, respectively, and 84.8% in the laser group). The adverse events that occurred in ≥10% of the patients are listed in Table 4. The majority of AEs were considered to be unrelated to study treatment or the injection/procedure.

The number of patients who were discontinued due to AEs was five (10.9%), seven (15.2%), seven (15.2%), and four (8.7%) in the PF-04523655 0.4-mg, 1-mg, and 3-mg groups and the laser photocoagulation group, respectively. The most common events reported included postinjection IOP increased in the study eye, systemic hypertension, conjunctival hemorrhage of the study eye, retinal hemorrhage of the study eye, and nasopharyngitis. The high rates of increased IOP in the PF-04523655 groups may have been due to the 100 μL volume of the intravitreal injections and/or the criterion in the study protocol, which required investigators to report any observations of postinjection IOP increase >5 mm Hg that occurred 1 hour postinjection as AEs.

There were two cases of mild posterior subcapsular cataracts related to PF-04523655. Both patients continued to receive PF-04523655 without progression of the posterior subcapsular cataracts, and both patients stayed in the study until the study was terminated.

Overall, the treatment-emergent serious adverse events (SAEs) experienced by patients in this study were typical of events observed for patients of this age with diabetes mellitus and DME. A total of eight (17.4%), 12 (26.1%), 17 (37.0%), and 10 (21.7%) subjects in the PF-04523655 0.4-mg, 1-mg, 3-mg groups and the laser group reported SAEs, respectively. None of the SAEs were considered related to study treatment. Three patients had SAEs related to the injection procedure and were discontinued from the study. One patient developed transient loss of vision following the intravitreal injection. Another was diagnosed with endophthalmitis 3 days following the patient's first intravitreal injection of 0.4 mg of PF-04523655. This patient had an emergency vitrectomy with intraocular injection of antibiotics and steroids. The eye was eviscerated 1 ½ weeks later because of increasing pain with no light perception. The patient had a HbA1c = 7.4%, normal serum creatinine level, and no proteinuria at baseline. Approximately 6 months later, at a different investigational site and with a different lot of PF-04523655, another patient developed endophthalmitis 3 days after the sixth intravitreal injection of 3-mg PF-04523655. This patient was treated successfully with topical antibiotics and steroids. This patient had an elevated HbA1c = 9.7% with an elevated serum creatinine = 1.5 mg/dL and 2+ proteinuria.

The percentage of patients with systolic BP >120 mm Hg or diastolic BP >80 mm Hg was higher posttreatment and was similar among treatment groups. Relevant laboratory abnormalities without regard to baseline findings for each of the

Table 3. Percentages of Patients with Improvement or Deterioration in BCVA at Month 12 (LOCF), Study Eye, ITT Population

	PF-04523655			
	0.4 mg $N = 46$	**1 mg** $N = 46$	**3 mg** $N = 46$	**Laser Photocoagulation** $N = 46$
Loss ≥15 letters, *n* (%)	3 (6.5)	1 (2.2)	2 (4.3)	1 (2.2)
Loss ≥10 to <15 letters, *n* (%)	1 (2.2)	0	0	1 (2.2)
Loss ≥5 to <10 letters, *n* (%)	5 (10.9)	8 (17.4)	3 (6.5)	3 (6.5)
Change with +/− 4 letters, *n* (%)	22 (47.8)	14 (30.4)	14 (30.4)	26 (56.5)
Gain ≥5 to <10 letters, *n* (%)	5 (10.9)	9 (19.6)	14 (30.4)	7 (15.2)
Gain ≥10 to <15 letters, *n* (%)	6 (13.0)	9 (19.6)	5 (10.9)	7 (15.2)
Gain ≥15 letters, *n* (%)	4 (8.7)	5 (10.9)	8 (17.4)	1 (2.2)
15-letter gainer* proportion difference from laser, %	6.5	8.7	15.2	
[90% CI], %	[−1.2, 14.2]	[0.4, 17.0]	[5.4, 25.1]	
10-letter gainer† proportion difference from laser, %	4.3	13.0	10.9	
[90% CI], %	[−9.2, 17.9]	[−1.4, 27.5]	[−3.4, 25.1]	
15-letter loser‡ proportion difference from laser, %	4.3	0.0	2.2	
[90% CI], %	[−2.6, 11.3]	[−5.0, 5.0]	[−3.9, 8.3]	

* 15-letter gainer: patients who gained ≥15 letters in BCVA compared to baseline.
† 10-letter gainer: patients who gained ≥10 letters in BCVA compared to baseline.
‡ 15-letter loser: patients who lost ≥15 letters in BCVA compared to baseline.

treatment groups (PF-0423655 0.4 mg, 1 mg, 3 mg, and focal/grid laser) were elevated HbA1c (63%, 57%, 59%, and 67%, respectively); hyperglycemia (37%, 60%, 55%, and 44%, respectively); elevated serum creatinine (29%, 26%, 25%, and 21%, respectively); elevated blood urea nitrogen (27%, 19%, 27%, and 28%); and proteinuria (23%, 21%, 44%, and 14%, respectively).

Discussion

This multicenter clinical trial of the safety and efficacy of PF-04523655 is the first study investigating siRNA as a therapeutic approach for DME. At doses up to 3 mg, PF-04523655 demonstrated minimal efficacy compared with focal/grid laser photocoagulation and at the 3- and 6-month interim analyses, there was an increased discontinuation rate in the PF-04523655 treatment groups compared with the focal/grid laser photocoagulation group. The study was not terminated at either of these interim analyses because of a general trend for improved BCVA with time. However, the study was terminated on December 17, 2010, following the third interim analysis, based on the continued high patient discontinuation rate and the moderate improvement in BCVA. At the time of study termination, only eight patients completed the 24-month study visit and no patient completed the 36-month end-of-study visit.

Since focal/grid laser was the current standard of care for DME, rescue criteria were only enforced in the PF-04523655 groups and not in the laser group. PF-04523655 treated patients meeting the rescue criteria were discontinued from the study. At month 12, the discontinuation rates in the PF-04523655 groups were all substantially higher than the laser

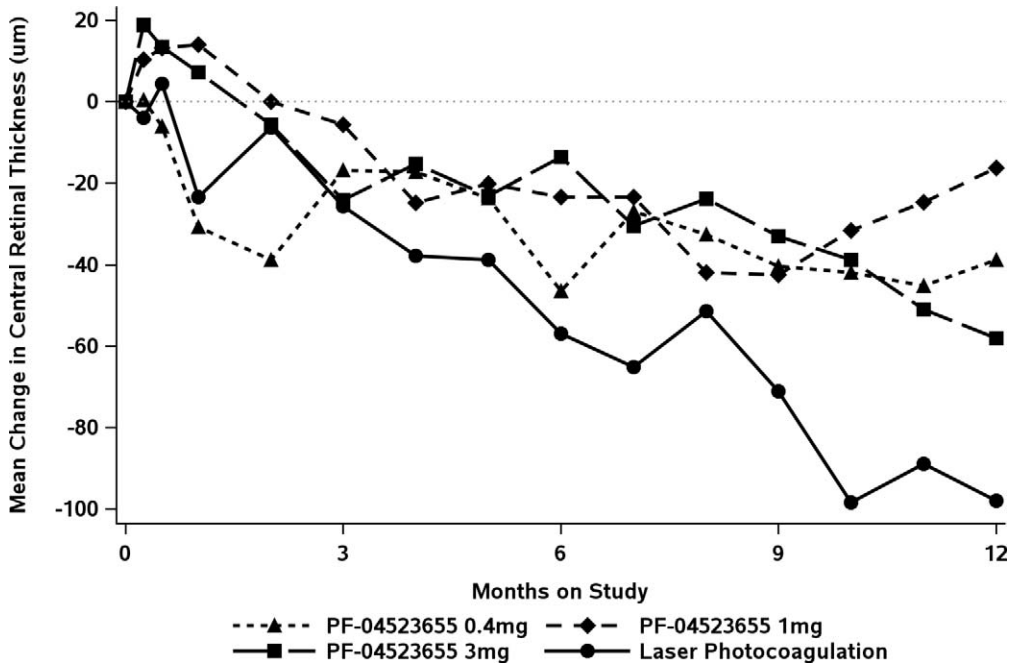

Figure 3. Mean change in retinal central subfield thickness (μm) from baseline (LOCF) by study visit, study eye, ITT population.

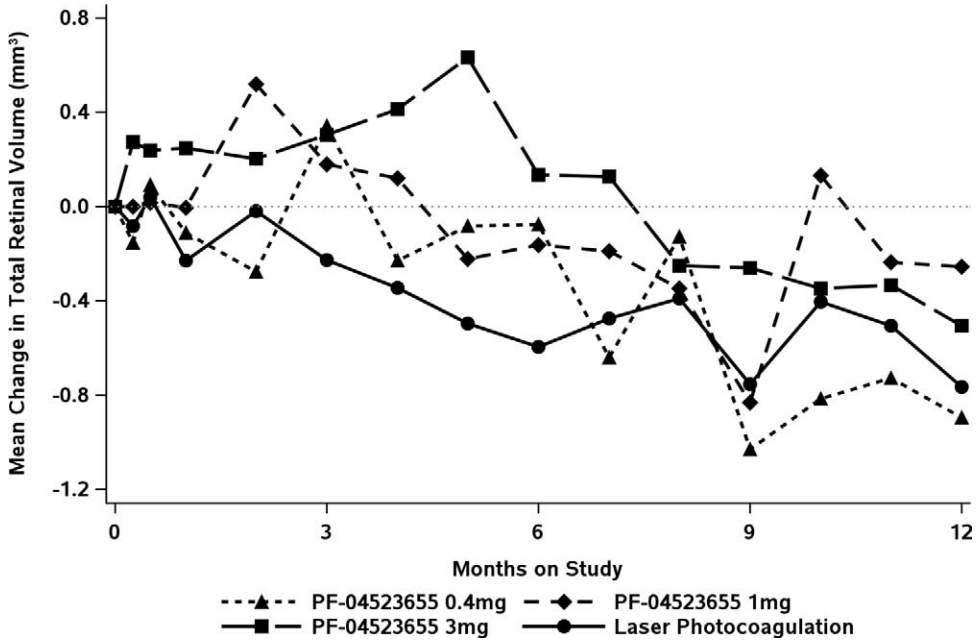

Figure 4. Mean change in total retinal volume (mm³) from baseline (Observed) by study visit, study eye, ITT population.

photocoagulation group and were inversely related to dose levels. The discontinuation rate for patients receiving PF-04523655 3 mg was approximately 40% less than for the 0.4-mg dose.

All three dose levels of PF-04523655 continued to improve visual acuity from baseline through month 12 in patients with DME. The improvement in BCVA from baseline occurred within the first month after the intravitreal injection of PF-04523655 and by the 6-month visit, there was a dose response that was maintained until month 12 (Fig. 2). Both the 1-mg and 3-mg doses had greater mean gains in visual acuity than laser therapy. There was also a positive trend between PF-04523655 dose level and the proportion of patients gaining $\geq$15 letters.

At month 12, the PF-04523655 3-mg group (5.77 letters) showed a trend for greater improvement in BCVA from baseline than the laser photocoagulation group (2.39 letters) ($P = 0.08$; 2-sided $\alpha = 0.10$). Since this was not a confirmatory study, the lower P value served as a signal for trends in the study to assist with making internal decisions concerning further clinical development with higher dosing of the compound.

By the month-3 visit, all doses of PF-04523655 reduced mean central subfield thickness, which was maintained until termination of the study. At month 12, the mean reduction in central subfield thickness with the PF-04523655 3-mg dose was greater than the other doses, but still was approximately 50%

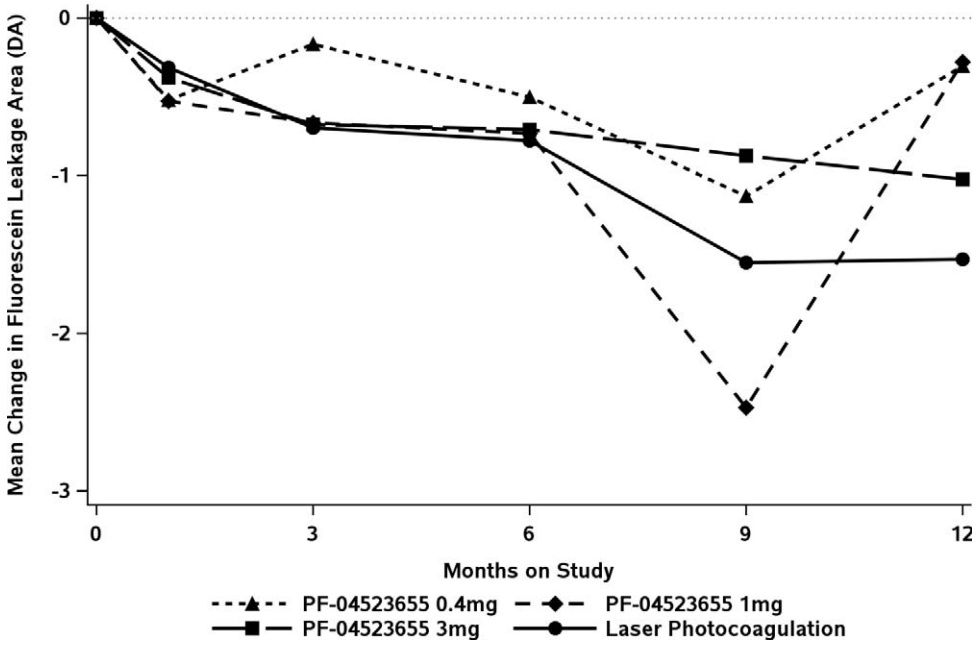

Figure 5. Mean change in fluorescein leakage area within grid (DA) from baseline (Observed) by study visit, study eye, ITT population.

Table 4. AEs Occurring in ≥10% of the Patients in Any Treatment Group

Adverse Event	PF-04523655 0.4 mg *N* = 46	PF-04523655 1 mg *N* = 46	PF-04523655 3 mg *N* = 46	Laser Photocoagulation *N* = 46
Blood creatinine increased, *n* (%)		6 (13.0)	3 (6.5)	2 (4.3)
Conjunctival hemorrhage, (study eye), *n* (%)	8 (17.4)	6 (13.0)	5 (10.9)	2 (4.3)
Fall	1 (2.2)	1 (2.2)	1 (2.2)	5 (10.9)
Glycosylated hemoglobin increased, *n* (%)	1 (2.2)	5 (10.9)	2 (4.3)	3 (6.5)
Hypertension	4 (8.7)	7 (15.2)	9 (19.6)	4 (8.7)
Intraocular pressure increased (fellow eye), *n* (%)	2 (4.3)	1 (2.2)	6 (13.0)	3 (6.5)
IOP increased (study eye, post IVT*), *n* (%)	18 (39.1)	20 (43.5)	24 (52.2)	1 (2.2)
Retinal hemorrhage (fellow eye), *n* (%)		1 (2.2)	5 (10.9)	2 (4.3)
Retinal hemorrhage (study eye), *n* (%)	4 (8.7)	2 (4.3)	5 (10.9)	3 (6.5)

* IVT = intravitreal injection of PF-04523655.

less than the laser group (Fig. 3). There was no evidence that the changes in retinal central subfield thickness, macular volume, fluorescein leakage area, or diabetic retinopathy related to the dose of PF-04523655. The lack of correlation between the change in these DME structural measures and PF-04523655 dose suggests that PF-04523655 may be working through a mechanism that does not involve vascular permeability. One possibility is PF-04523655 increases pigment epithelial derived factor (PEDF) with suppression of the RTP 801 gene (Pfizer; data on file). PEDF increases the expression of glutamine synthetase in retinal Müller cells, which is associated with a decrease in diabetic retinopathy.[21] In addition, PF-04523655 increased survival of retinal ganglion cells in rats following optic nerve crush (O'Neil JT, et al. *IOVS* 2012;53:ARVO E-Abstract 5139). The potential neuroprotective attributes of PF-042345655 may explain the improved BCVA in the study patients in the absence of anatomical changes.

Treatment with intravitreal PF-04523655 was generally safe and well-tolerated, with very few AEs that were considered treatment related. The majority of AEs were mild or moderate in severity. There were two deaths (cardiac arrest and aspiration pneumonia) and both were not treatment related. A total of 47 (26%) patients experienced SAEs and all were not treatment related. This high incidence of SAEs is common in the diabetic population and has been seen in other studies.[22] The most common of these SAEs were cardiac disorders as expected in this diabetic population. There were two cases of endophthalmitis in the PF-04523655 groups attributed to the injection procedure.

In this study, there was no evidence that PF-04523655 was locally or systemically proinflammatory, which is consistent with the findings of preclinical nonhuman primate studies (Pfizer; data on file). The lack of inflammatory response to PF-04523655 implies that it does not stimulate off-target toll like receptor 3 (TLR3) pathways.[23,24]

Laser focal/grid therapy has been the standard of care for the treatment of diabetic macular edema.[1] Multiple VEGF antagonists, such as ranibizumab, bevacizumab, pegaptanib, and aflibercept (VEGF Trap-Eye), have significantly improved visual acuity and reduced central retinal thickness more than laser focal/grid laser therapy in patients with DME.[7–9,15–18] These studies, along with the present study of PF-04523655, suggest that medical therapy may become an alternative to focal/grid laser therapy for the treatment of DME. Since PF-04523655 showed a dose-related tendency for improvement in BCVA and there were no dose limiting toxicities, studies of higher doses are planned to determine the optimal efficacious dose of PF-04523655. In view of the regulatory approval of anti-VEGF therapy for the treatment of diabetic macular edema in the United States and Europe, the next trial will compare the efficacy of higher doses of PF-04523655 to ranibizumab.

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Appendix

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