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VX-659–Tezacaftor–Ivacaftor in Patients with Cystic Fibrosis and One or Two Phe508del Alleles

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ABSTRACT

BACKGROUND

The next-generation cystic fibrosis transmembrane conductance regulator (CFTR) corrector VX-659, in triple combination with tezacaftor and ivacaftor (VX-659–tezacaftor–ivacaftor), was developed to restore the function of Phe508del CFTR protein in patients with cystic fibrosis.

METHODS

We evaluated the effects of VX-659–tezacaftor–ivacaftor on the processing, trafficking, and function of Phe508del CFTR protein using human bronchial epithelial cells. A range of oral VX-659–tezacaftor–ivacaftor doses in triple combination were then evaluated in randomized, controlled, double-blind, multicenter trials involving patients with cystic fibrosis who were heterozygous for the Phe508del *CFTR* mutation and a minimal-function *CFTR* mutation (Phe508del–MF genotypes) or homozygous for the Phe508del *CFTR* mutation (Phe508del–Phe508del genotype). The primary end points were safety and the absolute change from baseline in the percentage of predicted forced expiratory volume in 1 second (FEV_1).

RESULTS

VX-659–tezacaftor–ivacaftor significantly improved the processing and trafficking of Phe508del CFTR protein as well as chloride transport in vitro. In patients, VX-659–tezacaftor–ivacaftor had an acceptable safety and side-effect profile. Most adverse events were mild or moderate. VX-659–tezacaftor–ivacaftor resulted in significant mean increases in the percentage of predicted FEV_1 through day 29 ($P<0.001$) of up to 13.3 points in patients with Phe508del–MF genotypes; in patients with the Phe508del–Phe508del genotype already receiving tezacaftor–ivacaftor, adding VX-659 resulted in a further 9.7-point increase in the percentage of predicted FEV_1. The sweat chloride concentrations and scores on the respiratory domain of the Cystic Fibrosis Questionnaire–Revised improved in both patient populations.

CONCLUSIONS

Robust in vitro activity of VX-659–tezacaftor–ivacaftor targeting Phe508del CFTR protein translated into improvements for patients with Phe508del–MF or Phe508del–Phe508del genotypes. VX-659 triple-combination regimens have the potential to treat the underlying cause of disease in approximately 90% of patients with cystic fibrosis. (Funded by Vertex Pharmaceuticals; VX16-659-101 and VX16-659-001 ClinicalTrials.gov numbers, NCT03224351 and NCT03029455.)

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Cystic fibrosis is a serious, autosomal-recessive, multisystem disease that affects approximately 80,000 people worldwide and is associated with early death due to progressive lung disease.[1,2] Cystic fibrosis is caused by diminished quantity and defective function of an epithelial anion channel, the cystic fibrosis transmembrane conductance regulator (CFTR), as a consequence of mutations in *CFTR*.[3,4] For approximately 60% of patients with cystic fibrosis (i.e., those with two Phe508del alleles or a gating or residual-function *CFTR* mutation), small-molecule CFTR modulators provide therapeutic benefit by restoring CFTR activity.[5] However, high levels of chloride-transport restoration have not been achieved for the majority of patients with cystic fibrosis, approximately 90% of whom are either homozygous or heterozygous for the Phe508del *CFTR* mutation.[6] There are currently no approved CFTR modulators to treat the estimated 30% of patients who are heterozygous for the Phe508del *CFTR* mutation and a minimal-function *CFTR* mutation (Phe508del–MF genotypes), which leaves a substantial portion of the patient population without a treatment addressing the basic defect.[7-9]

The Phe508del *CFTR* mutation causes severe processing and trafficking defects, which result in decreased quantity and function of CFTR protein at the cell surface,[4] and has proven to be a challenging molecular target. Among patients who have at least one Phe508del *CFTR* mutation, approximately half have a second Phe508del allele (Phe508del–Phe508del genotype), and one third have a minimal-function *CFTR* mutation as the second allele. Minimal-function mutations, which produce either no protein or a defective protein that is unresponsive to CFTR modulators, include nonsense mutations, insertion and deletion mutations, canonical splicing mutations, and certain severe protein misfolding mutations.[1] A CFTR modulator regimen that could achieve high levels of functional CFTR in patients with a Phe508del allele, regardless of whether the second *CFTR* allele is also responsive to CFTR modulator therapy (e.g., a second Phe508del allele) or is incapable of response (i.e., the allele contains a minimal-function mutation), would be of value.

On the basis of the well-established understanding of the molecular defects caused by the Phe508del *CFTR* mutation, two complementary approaches have been developed to restore chloride transport by increasing the quantity and function of Phe508del CFTR protein at the cell surface. CFTR correctors, such as lumacaftor and tezacaftor, bind the Phe508del CFTR protein, augmenting intracellular processing and trafficking and thereby increasing the amount of mature CFTR available at the cell surface. The CFTR potentiator ivacaftor increases the channel-gating activity of Phe508del CFTR protein that is delivered to the cell surface, to augment anion transport.[10] With differing mechanisms of action, the combination of CFTR correctors and a CFTR potentiator is required to increase both the amount and function of Phe508del CFTR protein and is more effective than either approach alone.[10]

For patients with the Phe508del–Phe508del genotype, dual combinations of a first-generation CFTR corrector (lumacaftor or tezacaftor) and ivacaftor provide both short-term and long-term clinical benefit and are now accepted as the standard of care.[11] Nonetheless, these dual combinations do not fully restore function to Phe508del CFTR protein and are not sufficiently active to improve outcomes in patients with Phe508del–MF genotypes. As a consequence, there remains a need for highly effective CFTR modulation that will more adequately treat the underlying cause of disease in most patients with cystic fibrosis.[12-14]

We have previously shown in vitro that a combination of two correctors with distinct binding sites on CFTR and complementary mechanisms of action can increase the amount of Phe508del CFTR protein at the cell surface to a greater extent than either corrector alone.[15] On the basis of these findings, we developed next-generation CFTR correctors for use in combination with tezacaftor–ivacaftor to increase both the amount and function of Phe508del CFTR protein to a greater extent than a dual combination of a corrector and a potentiator. VX-659, a next-generation corrector, has both a different structure and a different mechanism of action. Its distinct mechanism of action is based on clear additivity on functional and biochemical assays of CFTR processing, trafficking, and function in vitro. Additivity of VX-659 to tezacaftor and ivacaftor in vitro increases Phe508del CFTR protein processing within the cell and trafficking to the cell surface. VX-659 shares some structural similarity and a mechanism of action with the next-generation corrector VX-445, described in a companion report also appearing in the *Journal*.[16] Because tezacaftor and VX-659 work through different mechanisms, we hypothesized that the

combination of VX-659 and tezacaftor would increase the amount of Phe508del CFTR protein at the cell surface more than either compound alone, an effect that could be potentiated by ivacaftor to further increase chloride transport.

We evaluated the effect of the VX-659–tezacaftor–ivacaftor triple combination on the processing, trafficking, and function of Phe508del CFTR protein in airway epithelial cells, which are an important and predictive preclinical model in cystic fibrosis.[10] These cells were obtained from patients with Phe508del–MF or Phe508del–Phe508del genotypes. We then conducted a small, phase 1 trial involving patients, followed by a larger, phase 2, proof-of-concept trial to evaluate the safety and efficacy of a range of VX-659 triple-combination doses in patients with one or two Phe508del alleles. For patients with Phe508del–MF genotypes, there is no available CFTR modulator, whereas for patients with the Phe508del–Phe508del genotype the standard of care includes a two-drug CFTR modulator combination; as a consequence, the trial designs differed according to patient genotype.

METHODS

PRECLINICAL DEVELOPMENT

The in vitro pharmacologic characteristics of VX-659 were evaluated in biochemical and functional studies alone and in dual and triple combination with tezacaftor–ivacaftor with the use of human bronchial epithelial cells derived from patients with cystic fibrosis and Phe508del–MF genotypes (four donors) or the Phe508del–Phe508del genotype (three donors). For details, see the Methods section in the Supplementary Appendix (available with the full text of this article at NEJM.org).

CLINICAL DEVELOPMENT

Trial Design and Oversight

A randomized, placebo-controlled, double-blind, multicenter, phase 1 trial (VX16-659-001) was conducted to evaluate preliminary pharmacokinetics and initial safety in a small group of patients with cystic fibrosis (Fig. 1). This trial enrolled patients 18 years of age or older with cystic fibrosis and Phe508del–MF *CFTR* genotypes and was conducted at nine sites in the United Kingdom from April 2017 through July 2017. Patients were randomly assigned, in a 2:1 ratio, to receive 14 days of active treatment with oral VX-659 at a dose of 120 mg every 12 hours in combination with tezacaftor (100 mg once daily) and ivacaftor (150 mg every 12 hours) or to receive oral triple placebo in parallel cohorts. Triple placebo was used as the control in this and subsequent trials involving patients with Phe508del–MF genotypes because these patients currently lack an approved standard-of-care CFTR modulator regimen.

After the phase 1 trial, a randomized, parallel-track, placebo- or active-controlled, double-blind, multicenter, dose-ranging, phase 2 trial (VX16-659-101) was conducted with multiple dose levels of VX-659 (Fig. 1). This trial enrolled patients 18 years of age or older with cystic fibrosis and Phe508del–MF or Phe508del–Phe508del *CFTR* genotypes. The phase 2 trial was conducted at 48 sites in the United States, the United Kingdom, Ireland, and Israel from August 2017 through February 2018.

The VX16-659-101 trial had three parts. In the first part, patients with Phe508del–MF genotypes were assigned to receive 4 weeks of active treatment with oral VX-659 at doses of 80, 240, or 400 mg once daily in triple combination with tezacaftor (100 mg once daily) and ivacaftor (150 mg every 12 hours) or to receive oral triple placebo in parallel cohorts. A randomization ratio of 1:2:2:1 was used to decrease the variability and thus increase the accuracy of the within-group efficacy estimate for the intermediate- and high-dose levels, at which the efficacy was predicted to plateau. This intervention period was followed by 4 days of only tezacaftor–ivacaftor (washout of VX-659) for those who had received active triple-combination therapy.

In the second part of the trial, patients with the Phe508del–Phe508del genotype were recruited. Because dual-combination CFTR modulators are the standard of care for these patients, tezacaftor–ivacaftor was used as the active control. Patients received 4 weeks of oral tezacaftor–ivacaftor during a run-in period, followed by randomization (in a 2:1 ratio) to 4 weeks of either oral VX-659 (400 mg once daily) or matched placebo in combination with tezacaftor–ivacaftor. This intervention period was followed by 4 weeks of tezacaftor–ivacaftor only (washout of VX-659). Baseline status for efficacy end points was assessed at the time of randomization, when all patients with the Phe508del–Phe508del genotype had been receiving daily tezacaftor–ivacaftor for at least 28 days.

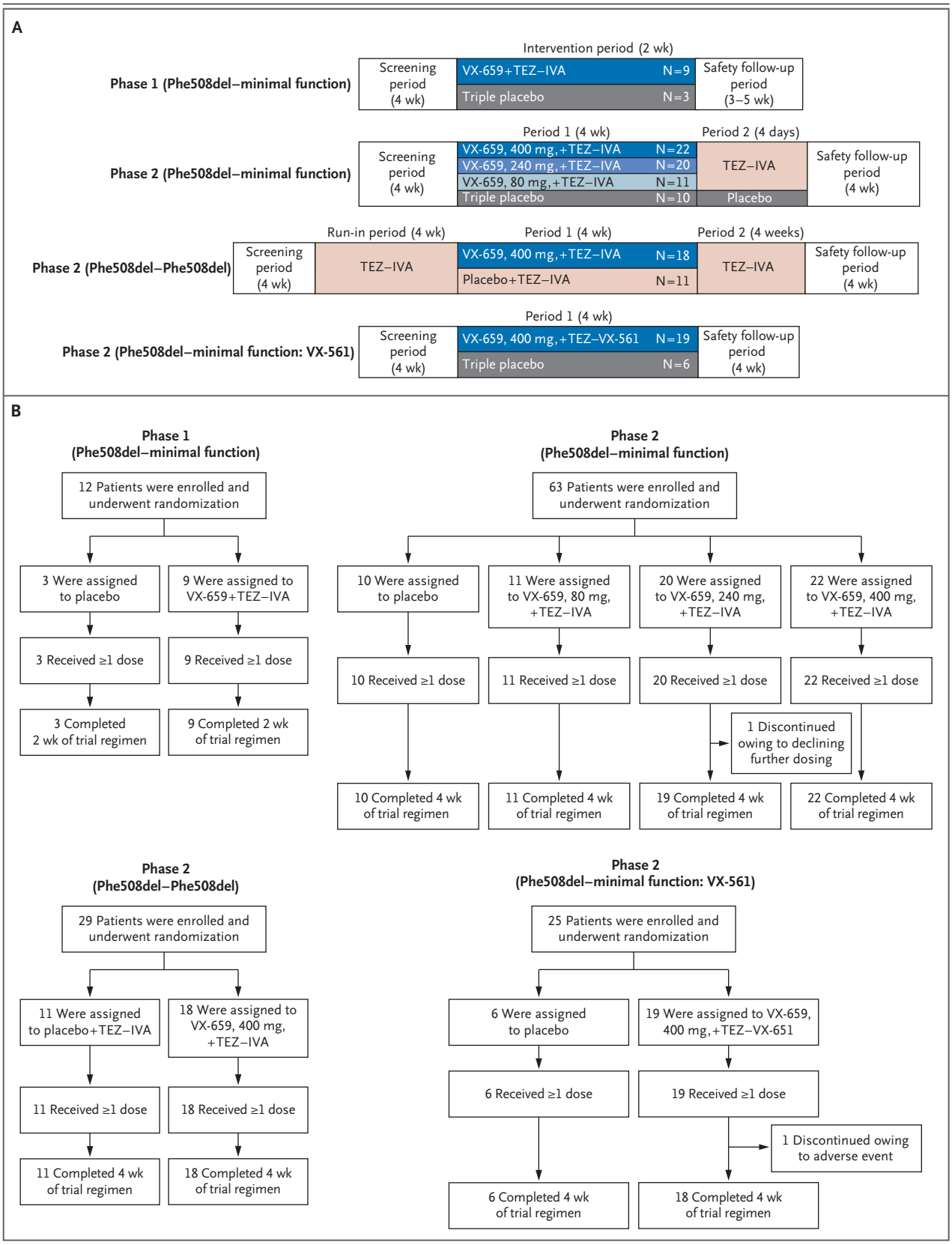


Figure 1 (facing page). Trial Design and Randomization and Follow-up of Patients.

Panel A shows the design of the phase 1 trial (VX16-659-001) and phase 2 trial (VX16-659-101) involving patients who were heterozygous for the Phe508del *CFTR* mutation and a minimal-function *CFTR* mutation (Phe508del–minimal function genotypes) and patients who were homozygous for the Phe508del *CFTR* mutation (Phe508del–Phe508del genotype). In the phase 1 trial, the dose of VX-659 was 120 mg every 12 hours; tezacaftor–ivacaftor (TEZ–IVA) was administered at a dose of 100 mg of TEZ once daily and 150 mg of IVA every 12 hours. In the phase 2 trial, the dose of VX-659 shown is the dose that was administered once daily; TEZ–IVA was administered at the same dose as in the phase 1 trial. A subgroup of patients with the Phe508del–minimal function genotype received VX-561 once daily instead of IVA every 12 hours. In the phase 2 trial, period 1 is the 4-week intervention period; period 2 is a washout of VX-659, as applicable. Panel B shows the number of patients in each intervention group who received at least one dose of the trial regimen and the number who completed the intervention period of 2 weeks (phase 1 trial) or 4 weeks (phase 2 trial).

The third part of the VX16-659-101 trial involved patients with Phe508del–MF genotypes who received VX-659–tezacaftor–VX-561 or triple placebo. VX-561 is a deuterated form of the CFTR potentiator ivacaftor that is administered orally once daily; the nondeuterated form of ivacaftor is administered twice daily. For details, see the Methods section in the Supplementary Appendix.

For both the VX16-659-001 and VX16-659-101 trials, the trial protocols and informed-consent forms were approved by an independent ethics committee or institutional review board at each trial site. All enrolled patients provided written informed consent. Safety was monitored by an independent data and safety monitoring committee. The trials were designed by the sponsor, Vertex Pharmaceuticals, in collaboration with the authors. Data gathering and analysis were performed by Vertex Pharmaceuticals in collaboration with the authors and the VX16-659-001 and VX16-659-101 Study Groups. All the authors had full access to the trial data after the data were unblinded and provided critical review and input on the manuscript. The first draft of the manuscript was written by an author employed by the sponsor with the assistance of medical writers funded by the sponsor. Final decisions regarding the content of the submitted manuscript were made by the first and last authors, and all the authors made the decision to submit the manuscript for publication. The investigators vouch for the accuracy and completeness of the data generated at their respective institutions, and the investigators and Vertex Pharmaceuticals vouch for the fidelity of the trials to the protocols. Confidentiality agreements were in place between the sponsor and all investigators during the trials. Trial protocols, along with the statistical analysis plans, are available at NEJM.org (Protocol 1 [VX16-659-001] and Protocol 2 [VX16-659-101]).

Trial Participants

In addition to the characteristics described above, patients eligible for inclusion had a percentage of predicted forced expiratory volume in 1 second (FEV_1) of 40 to 90% at screening[17] and stable disease as determined by the investigator on the basis of clinical assessment, including cystic fibrosis symptoms and treatment during the 28 days before the first dose of the trial regimen. Inclusion and exclusion criteria and the equations used to determine each patient's percentage of predicted FEV_1 are provided in the Methods section in the Supplementary Appendix.

Phase 1 Trial Assessments

The primary end points were safety and side-effect profile, based on the assessment of adverse events, clinically significant laboratory-test results, standard 12-lead electrocardiograms, vital signs, and spirometric measurements.

Phase 2 Trial Assessments

The primary end points were safety, side-effect profile, and the absolute change in the percentage of predicted FEV_1 from baseline through day 29. Safety assessments included adverse events, clinical laboratory values, electrocardiograms, and vital signs. Summarized safety data were based on events observed during the interval from the first dose of the trial regimen to 28 days after the last dose of the trial regimen or the end of the trial, whichever occurred first. Secondary end points included the absolute change in sweat chloride concentration from baseline through day 29 and the absolute change from baseline in Cystic Fibrosis Questionnaire–Revised (CFQ-R) respiratory domain score at day 29. Scores range from 0 to 100, with higher scores indicating a higher patient-reported quality of life with re-

spect to respiratory status (minimal clinically important difference, 4 points).

STATISTICAL ANALYSIS

For both the phase 1 and 2 trials, the intention-to-treat population, which included all the patients who received at least one dose of the trial regimen and had the intended *CFTR* mutation, was used to analyze the efficacy outcomes. The safety population, which included all the patients who received at least one dose of the trial regimen, was used for all safety analyses. For the phase 1 trial, efficacy end points, including the absolute change from baseline in the percentage of predicted FEV_1 and sweat chloride concentration, were summarized with the use of descriptive statistics.

For the phase 2 trial, safety data from patients receiving VX-659–tezacaftor–ivacaftor who had Phe508del–MF or Phe508del–Phe508del genotypes were pooled for the safety analysis. The primary end point of the absolute change from baseline in the percentage of predicted FEV_1 was analyzed separately for each part of the trial with the use of a mixed-effects model for repeated measures, with the change from baseline in the percentage of predicted FEV_1 at days 15 and 29 as the dependent variable. The within-group estimate along with the 95% confidence intervals and the corresponding P values were calculated as the primary analysis. Secondary end points, including sweat chloride concentration and CFQ-R respiratory domain score, were analyzed similarly, and the within-group estimate and 95% confidence intervals were calculated. The widths of the confidence intervals have not been adjusted for multiple comparisons and the intervals should not be used to infer definitive treatment effects. The primary analysis for all efficacy end points was the within-group comparison. (Additional details of the statistical analysis are provided in the Methods section and Table S1 in the Supplementary Appendix.)

RESULTS

IN VITRO EFFICACY IN PRIMARY HUMAN BRONCHIAL EPITHELIAL CELLS

VX-659 improved the processing and trafficking of Phe508del CFTR protein in human bronchial epithelial cells derived from patients with Phe508del–MF or Phe508del–Phe508del genotypes, as shown by higher steady-state levels of mature CFTR protein than in controls (Fig. 2A and 2B). The combination of VX-659–tezacaftor without or with ivacaftor resulted in significantly higher steady-state levels of mature CFTR protein than either compound alone. Ivacaftor slightly reduced the magnitude of CFTR processing correction by VX-659–tezacaftor in human bronchial epithelial cells from patients with the Phe508del–Phe508del genotype; however, correction by the triple combination was greater than that observed after treatment with dual combinations of either tezacaftor–ivacaftor or VX-659–ivacaftor.

Untreated human bronchial epithelial cells from patients with Phe508del–MF or Phe508del–Phe508del genotypes showed minimal CFTR-mediated chloride transport, a finding consistent with little to no functional CFTR at the cell surface (Fig. 2C). Treatment with VX-659 alone produced a modest increase in chloride transport, which was further augmented by combination with tezacaftor, ivacaftor, or both. The greatest increase in chloride transport was observed with the triple combination of VX-659–tezacaftor–ivacaftor (Fig. 2C). Together, these in vitro studies provided the rationale for investigating a triple-combination regimen of two correctors, VX-659 and tezacaftor, with the CFTR potentiator ivacaftor in patients with cystic fibrosis with either Phe508del–MF or Phe508del–Phe508del genotypes.

VX-659—TEZACAFTOR—IVACAFTOR IN PATIENTS WITH CYSTIC FIBROSIS

Patient Population

As part of a phase 1 trial (VX16-659-001) in which safety and pharmacokinetics were initially shown in healthy volunteers, we evaluated preliminary safety and pharmacokinetics of VX-659–tezacaftor–ivacaftor in a cohort of 12 patients with cystic fibrosis with Phe508del–MF genotypes. Patients were randomly assigned to receive either VX-659–tezacaftor–ivacaftor (9 patients) or triple placebo (3 patients) (Fig. S1 and Tables S2 through S5 in the Supplementary Appendix).

In the subsequent phase 2 trial, 117 patients with cystic fibrosis and Phe508del–MF genotypes (88 patients) or the Phe508del–Phe508del genotype (29 patients) were randomly assigned to receive either a VX-659 triple-combination regimen containing ivacaftor (71 patients) or VX-561 (19 patients) or a control regimen (27 patients)

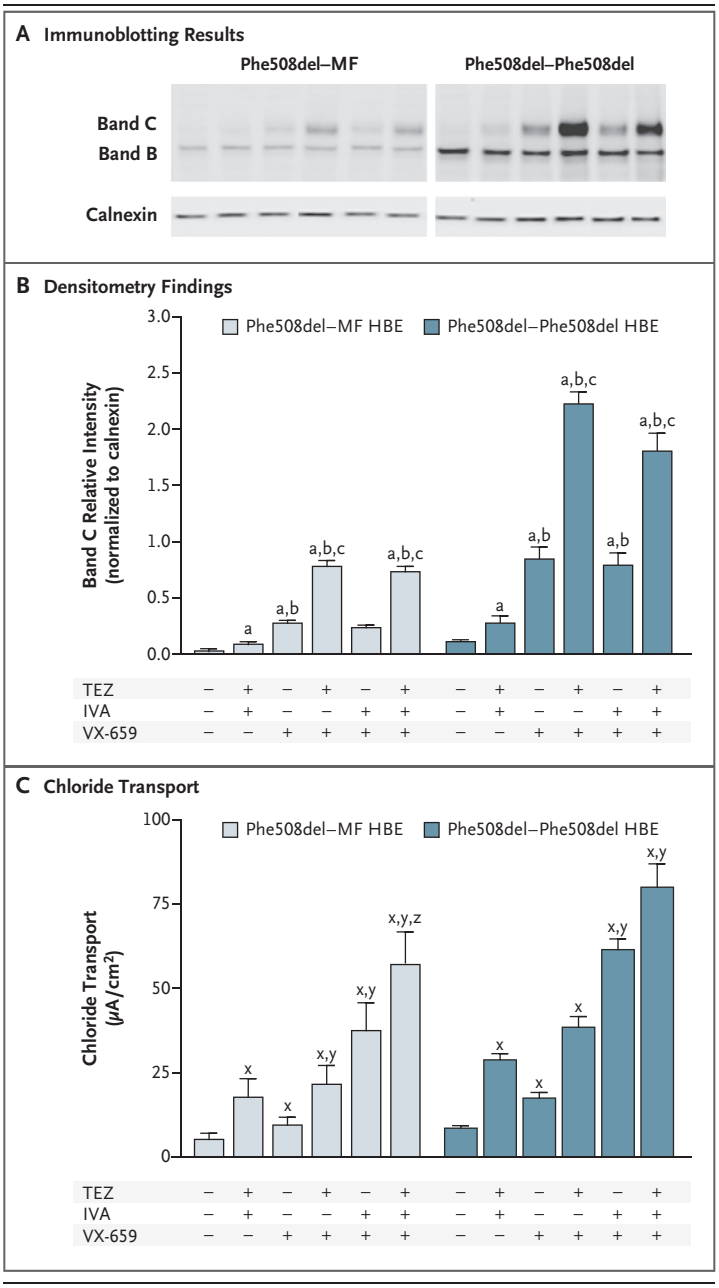


Figure 2. In Vitro Effect of VX-659 Alone or in Combination with TEZ, IVA, or TEZ–IVA.

Panels A and B show the effect of VX-659 on the processing and trafficking of Phe508del CFTR protein in human bronchial epithelial (HBE) cells derived from patients with Phe508del–minimal function (Phe508del–MF) or Phe508del–Phe508del genotypes. Panel A shows the results of representative immunoblotting from three independent experiments. Panel B shows the quantitative assessment of that data through densitometry findings pooled from three independent experiments, with six replicates in each experiment. Data are presented as mean relative intensities normalized to calnexin, a control for protein loading. The compound concentrations used were as follows: 1.4 μM of VX-659, 18 μM of TEZ, and 1 μM of IVA in the presence of 10 mg per milliliter of human serum albumin. The letter a represents $P<0.05$ for the comparison with vehicle, b $P<0.05$ for the comparison with TEZ–IVA, and c $P<0.05$ for the comparison with VX-659 in unpaired t-tests. Panel C shows an assessment of chloride transport in HBE cells treated with various combinations of TEZ (18 μM), IVA (1 μM), and VX-659 (1 μM) by means of an Ussing chamber. Data represent the mean of three or four donor bronchi, with three or four replicate experiments per donor. The letter x represents $P<0.05$ for the comparison with vehicle, y $P<0.05$ for the comparison with TEZ–IVA, and z $P<0.05$ for the comparison with VX-659–IVA in paired t-tests. In Panels B and C, T bars indicate standard errors.

(Fig. 1). A total of 115 patients completed the 4-week intervention period.

Baseline age, sex, percentage of predicted FEV_1, and sweat chloride concentration were generally well balanced across genotype and intervention groups (Table 1, and Table S6 [VX-659–tezacaftor–ivacaftor] and Table S7 [VX-659–tezacaftor–VX-561] in the Supplementary Appendix). For patients with Phe508del–MF genotypes who received VX-659–tezacaftor–ivacaftor or triple placebo, the mean baseline CFQ-R respiratory domain score was numerically higher in the placebo group than in the active-treatment groups (difference, 12.6 to 14.1 points). Consequently, analysis of the change in the CFQ-R respiratory domain score was performed both with and without adjustment for the baseline score (see the Methods section in the Supplementary Appendix for details).

Phase 1 Trial

The most common adverse events with VX-659–tezacaftor–ivacaftor were cough, infective pulmonary exacerbation of cystic fibrosis, and productive cough (each occurred in two patients). Oral candidiasis occurred in one patient in the VX-659–tezacaftor–ivacaftor group and one in the placebo group. Most adverse events were mild or moderate, and none led to interruption or discontinuation of the trial regimen. Two serious adverse events of infective pulmonary exacerbation of cystic fibrosis occurred in the VX-659–tezacaftor–ivacaftor group, one mild and one moderate in severity; both events occurred after the end of dosing (Table S3 in the Supplementary Appendix).

Table 1. Baseline Demographic and Clinical Characteristics of the Phe508del–Minimal Function and Phe508del–Phe508del Cohorts.*

Characteristic	Phe508del–Minimal Function				Phe508del–Phe508del†	
	Triple Placebo (N=10)	VX-659, 80 mg, +TEZ–IVA (N=11)	VX-659, 240 mg, +TEZ–IVA (N=20)	VX-659, 400 mg, +TEZ–IVA (N=22)	Placebo +TEZ–IVA (N=11)	VX-659, 400 mg, +TEZ–IVA (N=18)
Male sex — no. (%)	6 (60)	4 (36)	13 (65)	10 (45)	7 (64)	12 (67)
Age — yr	26.6±6.0	32.0±11.7	31.4±9.7	27.2±6.6	32.5±7.5	33.4±9.2
Percentage of predicted FEV_1	53.9±12.0	57.9±10.8	58.0±16.8	59.6±15.4	60.0±12.6	58.6±13.3
Sweat chloride — mmol/liter	98.2±13.3	102.7±7.0	100.5±9.0	100.7±11.6	96.6±11.4	91.9±11.6
CFQ-R respiratory domain score‡	77.2±15.1	63.1±18.5	64.4±17.8	64.6±20.7	65.7±17.4	68.5±14.1

* Plus–minus values are means ±SD. Patients with Phe508del–minimal function genotypes were heterozygous for the Phe508del *CFTR* mutation and a minimal-function *CFTR* mutation, and those with the Phe508del–Phe508del genotype were homozygous for the Phe508del *CFTR* mutation. All baseline characteristics were tested for balance between groups with the use of Fisher's exact test for categorical variables and the F test for all other variables. There were no significant differences between the intervention groups. The dose of VX-659 is the dose that was administered once daily. Tezacaftor–ivacaftor (TEZ–IVA) was administered at a dose of 100 mg of TEZ once daily and 150 mg of IVA every 12 hours. FEV_1 denotes forced expiratory volume in 1 second.
† The baseline characteristics of patients with the Phe508del–Phe508del genotype were assessed after a 4-week run-in with TEZ–IVA.
‡ Scores on the respiratory domain of the Cystic Fibrosis Questionnaire–Revised (CFQ-R) range from 0 to 100, with higher scores indicating a higher patient-reported quality of life with respect to respiratory status (minimal clinically important difference, 4 points).

VX-659–tezacaftor–ivacaftor therapy resulted in substantial improvements in the percentage of predicted FEV_1 and sweat chloride concentration (Fig. S1 and Table S5 in the Supplementary Appendix). These improvements were not seen in patients who received triple placebo.

Phase 2 Safety

Most patients who received VX-659–tezacaftor–ivacaftor (57 of 71 patients [80%]), triple placebo (9 of 10 [90%]), or tezacaftor–ivacaftor (9 of 11 [82%]) reported having at least one adverse event (Table 2, and Table S8 in the Supplementary Appendix). Among those receiving VX-659–tezacaftor–ivacaftor who had an adverse event, the maximum severity was mild or moderate for the majority of patients (53 of 57 patients [93%]). The most commonly observed adverse events (>10% occurrence in the pooled VX-659–tezacaftor–ivacaftor groups) were cough, infective pulmonary exacerbation of cystic fibrosis, headache, oropharyngeal pain, and increased sputum production. Four adverse events in patients receiving VX-659–tezacaftor–ivacaftor therapy were considered to be severe by site investigators (Table 2). Serious adverse events were reported in 7 patients (10%) who received VX-659–tezacaftor–ivacaftor, 3 patients (30%) who received triple placebo, and 2 patients (18%) who received tezacaftor–ivacaftor control. Most serious adverse events were infective pulmonary exacerbations of cystic fibrosis requiring hospitalization. No deaths occurred during the trial. There were no adverse events leading to discontinuation of the trial regimen in any patients who received VX-659–tezacaftor–ivacaftor.

One patient interrupted VX-659–tezacaftor–ivacaftor because of a rash. The rash resolved, and the patient resumed the trial regimen and completed the trial without recurrence of the rash. Two patients who received VX-659–tezacaftor–ivacaftor had elevations of alanine aminotransferase or aspartate aminotransferase levels (>3 to ≤5 times the upper limit of the normal range in one patient and >5 to ≤8 times the upper limit of the normal range in one patient) without concurrent elevation in the bilirubin level (Table S9 in the Supplementary Appendix). The elevations resolved during the trial without interruption of the trial regimen. There was no evidence of bronchoconstriction after VX-659 triple-combination dosing in any genotype or lung-function category as assessed by post-dose spirometry. The safety profile of VX-659–tezacaftor–VX-561 was similar to that of VX-659–tezacaftor–ivacaftor (Tables S10 and S11 in the Supplementary Appendix).

Phase 2 Efficacy

All doses of VX-659–tezacaftor–ivacaftor resulted in significant improvement in the percentage of predicted FEV_1 as compared with the respec-

Table 2. Summary of Adverse Events.

Event	Phe508del–Minimal Function				Phe508del–Phe508del		Any VX-659 (N=71)*
	Triple Placebo (N=10)	VX-659, 80 mg, +TEZ–IVA (N=11)	VX-659, 240 mg, +TEZ–IVA (N=20)	VX-659, 400 mg, +TEZ–IVA (N=22)	Placebo +TEZ–IVA (N=11)	VX-659, 400 mg, +TEZ–IVA (N=18)	
	number of patients (percent)						
Any adverse event†	9 (90)	10 (91)	15 (75)	17 (77)	9 (82)	15 (83)	57 (80)
Maximum severity of adverse event‡							
Mild	5 (56)	7 (70)	6 (40)	6 (35)	2 (22)	7 (47)	26 (46)
Moderate	4 (44)	3 (30)	8 (53)	10 (59)	4 (44)	6 (40)	27 (47)
Severe	0	0	1 (7)	1 (6)	3 (33)	2 (13)	4 (7)
Serious adverse event	3 (30)	1 (9)	4 (20)	1 (5)	2 (18)	1 (6)	7 (10)
Adverse event leading to interruption of the trial regimen	0	0	1 (5)	0	0	0	1 (1)
Adverse event leading to discontinuation of the trial regimen	0	0	0	0	0	0	0
Adverse events occurring in ≥5% of patients who received VX-659–TEZ–IVA							
Cough	1 (10)	3 (27)	6 (30)	4 (18)	2 (18)	4 (22)	17 (24)
Infective pulmonary exacerbation of cystic fibrosis	2 (20)	3 (27)	3 (15)	4 (18)	3 (27)	5 (28)	15 (21)
Headache	0	1 (9)	4 (20)	4 (18)	0	3 (17)	12 (17)
Oropharyngeal pain	0	0	3 (15)	4 (18)	0	2 (11)	9 (13)
Increased sputum production	0	2 (18)	1 (5)	3 (14)	1 (9)	3 (17)	9 (13)

* Shown are patients with Phe508del–minimal function or Phe508del–Phe508del genotypes who received VX-659.
† A patient with multiple events within a category was counted only once in that category.
‡ No events were considered life-threatening. The denominator is the number of patients with at least one adverse event.

Table 3. Absolute Change from Baseline in the Percentage of Predicted FEV_1 and Sweat Chloride Concentration through Day 29 and CFQ-R Respiratory Domain Score at Day 29.*

End Point	Phe508del–Minimal Function				Phe508del–Phe508del	
	Triple Placebo (N=10)	VX-659, 80 mg, +TEZ–IVA (N=11)	VX-659, 240 mg, +TEZ–IVA (N=20)	VX-659, 400 mg, +TEZ–IVA (N=22)	Placebo +TEZ–IVA (N=11)	VX-659, 400 mg, +TEZ–IVA (N=18)
Percentage of predicted FEV_1						
Absolute change from baseline	0.4±2.8	10.2±2.7	12.0±2.0	13.3±1.9	0.0±1.9	9.7±1.5
95% CI	–5.3 to 6.1	4.8 to 15.5	8.0 to 16.0	9.5 to 17.1	–3.9 to 3.9	6.6 to 12.7
P value†	0.90	<0.001	<0.001	<0.001	0.99	<0.001
Sweat chloride — mmol/liter						
Absolute change from baseline	2.9±4.6	–45.7±4.3	–43.8±3.4	–51.4±3.2	3.0±2.8	–42.2±2.2
95% CI‡	–6.3 to 12.2	–54.4 to –37.0	–50.7 to –37.0	–57.8 to –44.9	–2.8 to 8.9	–46.8 to –37.7
CFQ-R respiratory domain score§						
Absolute change from baseline	4.7±6.1	24.6±5.8	19.8±4.4	21.8±4.1	2.9±4.0	19.5±3.1
95% CI‡	–7.5 to 16.8	13.0 to 36.2	11.0 to 28.6	13.6 to 30.0	–5.2 to 11.1	13.1 to 25.9

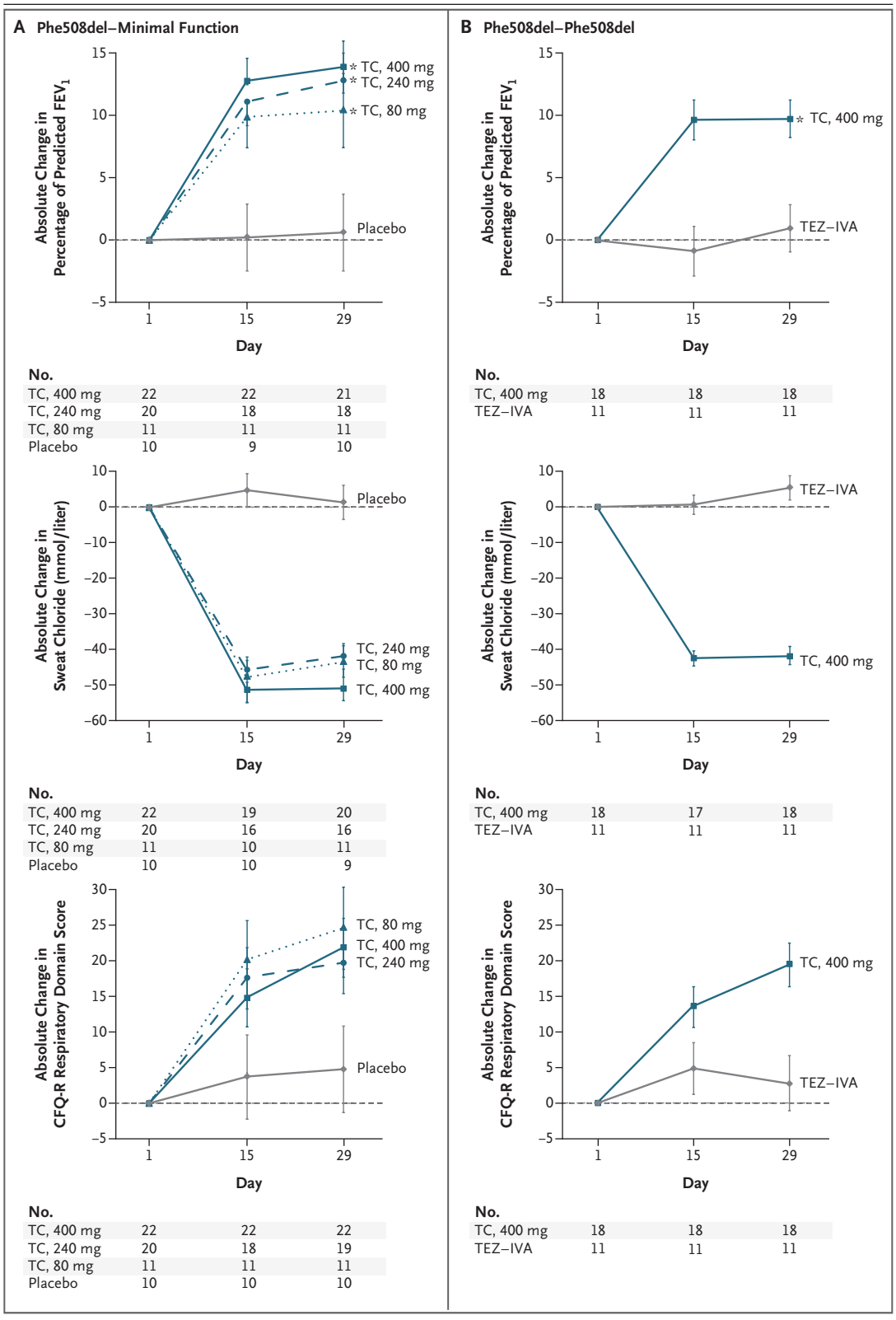
* Plus–minus values are least-squares means ±SE. Data were analyzed with the use of a mixed-effects model with repeated measures. CI denotes confidence interval.
† Shown is the within-group P value for the comparison with baseline.
‡ The widths of the confidence intervals have not been adjusted for multiple comparisons, and the intervals should not be used to infer definitive treatment effects.
§ Values for CFQ-R respiratory domain score were not adjusted for baseline score; a prespecified analysis with adjustment for baseline CFQ-R respiratory domain score is presented in Table S12 in the Supplementary Appendix.

tive within-group baseline values in patients with Phe508del–MF or Phe508del–Phe508del genotypes (Table 3). These improvements were observed at the first assessment on day 15 and were sustained at day 29 (Fig. 3). Changes indicating a beneficial effect of VX-659–tezacaftor–ivacaftor were observed for the secondary end points of sweat chloride concentration and the CFQ-R respiratory domain score, findings consistent with the magnitude of improvement in the percentage of predicted FEV_1 (Fig. 3 and Table 3). Improvement in the CFQ-R respiratory domain score at day 29 was observed with or without adjustment for the baseline score (Table 3 [without adjustment], and Table S12 in the Supplementary Appendix [with adjustment]). Data on the absolute and relative changes in FEV_1 as a percentage of the predicted value and in liters are provided in Table S12 in the Supplementary Appendix. Individual patient data for the absolute change from baseline in the percentage of predicted FEV_1 and sweat chloride concentration at day 29 are included in Figure S2 in the Supplementary Appendix. Similar improvements in all these end points were observed in patients with Phe508del–MF genotypes who received VX-659–tezacaftor–VX-561 (Table S13 and Fig. S3 in the Supplementary Appendix).

DISCUSSION

In our in vitro to in vivo studies, the next-generation CFTR corrector VX-659 in combination with

Figure 3 (facing page). Absolute Change from Baseline in the Percentage of Predicted FEV_1, Sweat Chloride Concentration, and CFQ-R Respiratory Domain Score.
Shown is the least-squares mean change in the percentage of predicted forced expiratory volume in 1 second (FEV_1) though day 29, sweat chloride concentration through day 29, and score on the respiratory domain of the Cystic Fibrosis Questionnaire–Revised (CFQ-R), without adjustment for baseline scores, at day 29 in patients with Phe508del–minimal function genotypes (Panel A) and those with the Phe508del–Phe508del genotype (Panel B). CFQ-R respiratory domain scores range from 0 to 100, with higher scores indicating a higher patient-reported quality of life with respect to respiratory status (minimal clinically important difference, 4 points). I bars indicate standard errors. Asterisks indicate a within-group P value of less than 0.001 for the comparison with baseline. TC denotes triple-combination therapy with VX-659–TEZ–IVA; the dose in milligrams represents the dose of VX-659 administered once daily.



tezacaftor–ivacaftor led to improvements in CFTR activity and in measures of lung function and quality of life in patients with cystic fibrosis and Phe508del–MF or Phe508del–Phe508del genotypes. In these phase 1 and phase 2 trials with intervention periods of 2 to 4 weeks, no dose-limiting side effects or toxic effects were noted with administration of VX-659. Most notably, patients with Phe508del–MF genotypes, who have only one responsive Phe508del allele and no currently available CFTR modulator therapy, had improvements in lung function, sweat chloride concentration, and CFQ-R respiratory domain score. In addition, patients with the Phe508del–Phe508del genotype who were already receiving tezacaftor–ivacaftor had additional improvements in these outcome measures after the administration of VX-659. Thus, these trials of VX-659–tezacaftor–ivacaftor pave the way for larger and longer trials of the clinical usefulness of triple-combination CFTR modulator regimens for patients with cystic fibrosis who have one or two copies of the Phe508del *CFTR* mutation.

Treatment with VX-659–tezacaftor–ivacaftor led to an average absolute increase in the percentage of predicted FEV_1 of up to 13.3 points in patients with Phe508del–MF genotypes, as compared with baseline values. In patients with the Phe508del–Phe508del genotype who were already receiving tezacaftor–ivacaftor, the addition of VX-659 resulted in an average absolute increase in the percentage of predicted FEV_1 of 9.7 points. The response with respect to lung function was largely apparent at the first assessment after the start of treatment and was sustained throughout the 4-week trial, findings consistent with the timing of responses observed with other effective CFTR modulator regimens.[12-14,18,19] The increases in the percentage of predicted FEV_1 were accompanied by improvements in sweat chloride concentration, an in vivo measure of CFTR function, and in the CFQ-R respiratory domain score, a patient-reported measure of respiratory symptoms.[12-14] Treatment with the alternative once-daily regimen of VX-659–tezacaftor–VX-561 led to similar improvements in patients with Phe508del–MF genotypes.

The clinical efficacy of VX-659–tezacaftor–ivacaftor that was noted in the phase 2 trial was clearly predicted by the in vitro cell-based bioassay. In vitro, we found that including all three components of the triple combination led to the largest effects on CFTR protein levels and chloride transport in cells from donors with either Phe508del–MF or Phe508del–Phe508del genotypes, with VX-659 adding substantially to the combined effect of tezacaftor and ivacaftor. Moreover, VX-659–tezacaftor–ivacaftor increased chloride transport in such cells more effectively than the dual combination of VX-659–ivacaftor and more than the individual agents alone. The magnitude of improvement in chloride transport in cells from patients with Phe508del–MF genotypes that were treated with VX-659–tezacaftor–ivacaftor reached approximately 50% of wild-type CFTR activity, an effect similar to that of ivacaftor in cells from patients with *CFTR* gating mutations.[20] On the basis of these data, the clinical effect of the triple-combination regimen in patients with at least one Phe508del allele was predicted to be similar to the effect of ivacaftor in patients with gating mutations,[18] and the subsequent clinical-trial results substantiated this prediction.

These trials of relatively short duration revealed no concerning safety signals across the genotype groups and range of VX-659 triple-combination doses studied. The majority of patients had adverse events that were mild or moderate in severity, and there were a limited number of interruptions or discontinuations of the trial regimen due to adverse events; no dose relationship was seen for these events. Overall, the safety profile of VX-659 triple-combination therapy was similar to that seen in previous studies of CFTR modulators.[12-14,18,19] In addition, two other components of the triple combination, tezacaftor and ivacaftor, have well-characterized safety profiles in short-term and long-term studies.[13,14,21,22]

In conclusion, we found that VX-659 triple-combination therapy led to improvements in all evaluated efficacy outcomes in patients with cystic fibrosis and Phe508del–MF or Phe508del–Phe508del genotypes. An additive response was seen for VX-659 triple-combination therapy in comparison with the dual combination of tezacaftor–ivacaftor in patients with the Phe508del–Phe508del genotype. These trials provide proof of the concept that targeting the Phe508del CFTR protein with a triple-combination corrector–potentiator regimen can restore CFTR function and has the potential to represent a clinical advance for patients with cystic fibrosis who harbor either one or two Phe508del alleles, approximately 9 of every 10 patients with the disease.

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APPENDIX

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