Nonsense (premature stop codon) mutations are causative in 5% to 15% of patients with monogenetic inherited disorders. PTC124, a 284-Dalton 1,2,4-oxadiazole, promotes ribosomal readthrough of premature stop codons in mRNA and offers therapeutic potential for multiple genetic diseases. The authors conducted 2 phase I studies of PTC124 in 62 healthy adult volunteers. The initial, single-dose study evaluated doses of 3 to 200 mg/kg and assessed fed-fasting status on pharmacokinetics following a dose of 50 mg/kg. The subsequent multiple-dose study evaluated doses from 10 to 50 mg/kg/dose twice per day (bid) for up to 14 days. PTC124 administered orally as a liquid suspension was palatable and well tolerated through single doses of 100 mg/kg. At 150 and 200 mg/kg, PTC124 induced mild headache, dizziness, and gastrointestinal events. With repeated doses through 50 mg/kg/dose bid, reversible transaminase elevations <2 times the upper limit of normal were sometimes observed. Immunoblot analyses of peripheral blood mononuclear cell extracts revealed no protein elongation due to nonspecific ribosomal readthrough of normal stop codons. PTC124 plasma concentrations exceeding the 2- to 10-µg/mL values associated with activity in preclinical genetic disease models were safely achieved. No sex-related differences in pharmacokinetics were seen. No drug accumulation with repeated dosing was apparent. Diurnal variation was observed, with greater PTC124 exposures after evening doses. PTC124 excretion in the urine was <2%. PTC124 pharmacokinetics were described by a 1-compartment model. Collectively, the data support initiation of phase II studies of PTC124 in patients with nonsense mutation–mediated cystic fibrosis and Duchenne muscular dystrophy.

Keywords: PTC124; nonsense mutation; genetic disease; phase I clinical trial; pharmacokinetics

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It is known that suppression of the nonsense mutation defect can be achieved with aminoglycoside antibiotics such as gentamicin. At high concentrations, binding of gentamicin to eukaryotic ribosomes induces the ribosomes to read through a premature stop codon within an mRNA molecule, inserting an amino acid and continuing translation to produce a complete protein. In mouse models of cystic fibrosis (CF) and Duchenne muscular dystrophy (DMD), as well as in pilot studies in human subjects with nonsense mutation–mediated CF or DMD, gentamicin has been shown to restore translation of full-length, functional CFTR or dystrophin, respectively. Although these results have served as a proof of concept for nonsense mutation suppression as a therapeutic approach to genetic disorders, serious dose-limiting renal and otic toxicities and the need for parental administration have precluded general use of gentamicin for this purpose.

Because of these limitations of gentamicin, there has been considerable interest in identifying safer and more conveniently administered compounds to treat patients with genetic disorders due to nonsense mutations. We conducted a high-throughput drug discovery program to specifically identify new agents that can permit suppression of disease-causing nonsense mutations. Employing cell-based and cell-free in vitro translation assays containing a signal protein modified to include a nonsense mutation, we discovered novel chemical structures that selectively induce ribosomal readthrough of premature stop codons in mRNA. Chemical and pharmacological optimization led to identification of PTC124 (3-[5-(2-fluorophenyl)-[1,2,4]oxadiazol-3-yl]-benzoic acid) (Figure 1), a 284-Dalton, orally bioavailable nonaminoglycoside compound as a candidate for further development.

Through in vitro characterization in translation assays, we have demonstrated that PTC124 induces dose- and time-dependent ribosomal readthrough of premature stop codons with greater potency than gentamicin. PTC124’s activity is selective; it does not permit ribosomes to read through normal stop codons in preclinical systems. In the same nonsense mutation–containing mouse models of CF and DMD used to evaluate gentamicin, we have shown that treatment with PTC124 promotes the production of full-length, functional CFTR and dystrophin, respectively. The CFTR protein is able to restore chloride channel activity, and the dystrophin is appropriately localized to muscle cell membranes and is functionally active, reducing muscle contraction injury and decreasing pathologic leakage of creatine kinase from muscles into the systemic circulation. Although precise exposure-response correlations have not been established, the nonclinical efficacy pharmacology data imply that achieving relatively continuous plasma concentrations above a target trough value of ~2 µg/mL may be associated with discernable readthrough activity, whereas achieving a trough value of ~10 µg/mL may be associated with maximal effects.

In support of clinical development of PTC124, we have performed comprehensive preclinical safety testing. In safety pharmacology studies in rats and dogs, we have demonstrated that oral administration of PTC124 induces no adverse neurological, pulmonary, or cardiovascular effects at doses through 1500 mg/kg. In toxicology studies in rats and dogs at oral doses through 1500 mg/kg for 28 days, PTC124 has shown good tolerability. In these studies, toxicokinetic exposure levels substantially exceeded the target concentrations required for nonsense suppression in preclinical efficacy pharmacology studies. We have also documented that PTC124 demonstrates virtually no human ether-à-go-go-related gene (hERG) channel inhibition, has no discernable antibiotic activity against gram-positive or gram-negative organisms, and is not mutagenic or genotoxic in standard in vitro and in vivo assays. PTC124 is stable when incubated with human liver microsomes.

Based on the available preclinical data, we are developing PTC124 as a therapy for patients with nonsense mutation–mediated CF and DMD. As a prelude to phase II clinical testing in patients with
these diseases, we performed the current phase I single-dose and multiple-dose studies in healthy volunteers to characterize the initial safety profile and palatability of PTC124, assess its pharmacokinetics, evaluate food effects, search for evidence of nonspecific normal stop codon readthrough, and define the most appropriate schedules for achieving target plasma concentrations in subsequent patient studies.

METHODS

We conducted both studies at a single center according to the principles of the current revision of the Declaration of Helsinki and good clinical practice (GCP) guidelines. A local institutional review board (Independent Investigational Review Board, Plantation, Fla) reviewed and approved the study protocols and the informed consent documents. Before any study-related procedures were performed, all subjects provided written informed consent for participation in the study.

Study Population

Entry criteria were comparable in both studies. Subjects were healthy male or healthy nonpregnant, nonlactating female volunteers ranging in age from 18 through 30 years and having a body mass index (BMI) ranging from 20 through 30 kg/m². Subjects were considered healthy based on medical history, physical examination, electrocardiogram (ECG), and clinical laboratory safety tests (including hematology, chemistry, and urinalysis assays, as well as hepatitis B, hepatitis C, and human immunodeficiency virus serology) performed within 21 days prior to initial study drug administration. In female subjects, serum pregnancy testing for β-human chorionic gonadotropin (β-HCG) was performed within 1 day before each study drug administration. Subjects with a history of severe adverse drug reactions or a history of drug or alcohol abuse were excluded from study participation. Specific periods of abstinence prior to initial study drug administration were required for tobacco (2 days); alcohol (7 days); predefined cytochrome P450 substrates, anticonvulsants, steroids, hormonal contraceptives, and anticoagulants (21 days); and investigational drugs (30 days). Patients were excluded if toxicology screens performed within 1 day prior to each study drug administration showed evidence of ethanol, cocaine, tetrahydrocannabinol, barbiturates, amphetamines, benzodiazepines, or opiates.

Study Design

The initial study was an escalating single-dose, alternating-panel, randomized, double-blind, placebo-controlled, safety, palatability, pharmacokinetic, and food effect study conducted in 2 stages. In stage 1, 16 subjects were to be enrolled into 2 panels (A or B) of 8 subjects each. Within each panel, subjects were to be randomly assigned to PTC124 or placebo (men: 3 drug, 1 placebo; women: 3 drug, 1 placebo). Six dose levels were to be evaluated: 3, 30, and 200 mg/kg in panel A and 10, 100, and 300 mg/kg in panel B. Subjects could therefore receive drug at as many as 3 dose levels with an interval of at least 2 weeks between doses. In stage 2, 12 additional subjects were to be randomized to receive 50 mg/kg of PTC124 after an overnight fast (3 men, 3 women) or within 30 minutes after consuming a standardized, high-fat, high-calorie meal (3 men, 3 women). One week later, subjects were to be crossed over to the opposite food intake regimen. The test meal comprised 2 eggs fried in butter, 2 strips of bacon, 2 slices of toast with butter, 4 ounces of hash brown potatoes, and 8 ounces of whole milk. In both stages, subjects were confined to the clinic on the evening before each study drug administration. They remained under observation for 72 hours and were to return for a follow-up visit 7 days after each study drug administration.

The subsequent study was an escalating multiple-dose, open-label, safety, and pharmacokinetic study. In stage 1, 24 subjects were enrolled in 4 cohorts of 6 subjects each (3 men, 3 women). The 4 cohorts underwent sequential dose escalations across 4 dose levels (10, 20, 30, and 50 mg/kg/dose administered twice per day [bid]). Subjects took the study medication with food for 7 days (with only the morning dose given on day 7). In stage 2, 6 additional subjects (3 men, 3 women) took PTC124 at 50 mg/kg/dose bid with food for 14 consecutive days. In both stages, subjects were confined to the clinic on the evening before the initial study drug administration. They remained under observation throughout the assigned dosing period and a 3-day follow-up period.

PTC124 Formulation and Administration

PTC124 drug substance was manufactured by Rhodia Pharma Solutions (Malvern, Pa), and the drug product was prepared by Patheon, Inc (Mississauga, Ontario, Canada) in accordance with current good manufacturing practices (cGMP). A matching placebo was provided in comparable packaging for the single-dose
study. PTC124 was provided as a powder for suspension, which was mixed with water shortly before dosing. In both studies, the PTC124 concentration in the suspension remained fixed at 150 mg/mL, whereas the dose volume was increased with increasing dose level. During the single-dose study, study drug was orally administered in the morning with additional water afterward such that the total liquid volume consumed was ~260 mL. Subjects were to avoid ingestion of any other substance except clear liquids or the designated meal (stage 2) within the period between 10 hours before and 4 hours after study drug administration. In the multiple-dose study, PTC124 suspended in water was orally administered every 12 hours, within 30 minutes after the start of the morning and evening meals; subjects also ingested lactose-free milk such that the total liquid volume consumed was ~240 mL.

**Safety and Palatability Assessments**

In both studies, data regarding adverse events, vital signs, blood counts, coagulation assessments, blood chemistry determinations, urinalyses, and ECGs were collected. Assessments were performed throughout the 72-hour confinement period and at the follow-up visit in the single-dose study, as well as throughout the 10- or 17-day confinement periods of the multiple-dose study. Drug palatability was assessed within 10 minutes after drug ingestion, on the first day of dosing in both studies and on the last day of dosing in the multiple-dose study, by administration of a questionnaire evaluating drug texture, taste, odor, and aftertaste.

**Evaluation of Nonspecific Stop Codon Readthrough**

In stage 1 of the single-dose study, blood samples to evaluate nonspecific stop codon readthrough effects were obtained from PTC124-treated subjects just before (0 hour) dosing and at 2, 4, 8, and 24 hours following dosing. Blood samples obtained at all 5 time points from the men and women receiving placebo were pooled by sex and used as additional untreated controls. During stage 2 of the multiple-dose study, blood samples were obtained from PTC124-treated subjects just before (0 hour) dosing on day 1, just before (0 hour) and at 3 and 12 hours after the morning dose on day 14, and at discharge on day 17. Peripheral blood mononuclear cells (PBMCs) and plasma were separated, frozen, and shipped to PTC Therapeutics. We pooled aliquots of PBMCs and plasma for PTC124-treated subjects by time point and sex. Pooled samples were loaded onto polyacrylamide gels optimized to obtain maximal separation between wild-type and any potential elongated readthrough protein products, subjected to electrophoresis, and transferred to nitrocellulose membranes for immunoblotting (Western blotting). Proteins evaluated included C-reactive protein (CRP), β2 microglobulin, and cystatin C. For CRP, purified human CRP was used as a wild-type marker. For β2 microglobulin and cystatin C, we were able to generate specific wild-type and elongated protein markers in vitro. In vitro–generated elongated protein markers were subjected to immunoblotting concurrently with the samples obtained from the clinical trial subjects. Standard molecular weight markers were also included on each gel. Immunoblotting was performed using primary antibodies specific for the proteins, with secondary antibodies comprising hors eradish peroxidase conjugates. For cystatin C, it was possible to generate a primary antibody that specifically recognized the elongated protein but not wild-type cystatin C.

As a positive control for the immunoblotting results, we also evaluated specific premature stop codon readthrough ex vivo in a cell-based reporter system. For this purpose, we assayed plasma samples obtained just before (0 hour) and at 2 and 8 hours following PTC124 administration in the single-dose study. Samples were pooled by sex and time point. Plasma was added to media supporting growth of cultured human embryonic kidney cells (HEK293 cells) that we had stably transfected with a firefly luciferase reporter gene containing a UGA premature stop codon at amino acid position 190.11 Cells were incubated for 20 hours and then assayed for chemoluminescence as a measure of drug-induced ribosomal readthrough of the premature stop codon in the luciferase protein.

**Blood and Urine Sample Collection and Determination of PTC124 Concentrations**

During the single-dose study, blood samples for determination of PTC124 concentrations were collected just before and at 0.25, 0.5, 1, 2, 3, 4, 6, 8, 12, 16, 24, 36, 48, and 72 hours after each study drug administration and once at the day 7 follow-up visit. In the dose escalation stage of the multiple-dose study, blood samples were collected on days 1 and 7 just before (0 hour) and at 0.25, 0.5, 1, 2, 3, 4, 6, 8, 10, and 12 hours after administration of the morning dose, as well as on days 2 to 6 just before (0 hour) and at 3...
hours after the morning dose, on day 8 at 24 hours after the last dose, and on day 10 before discharge from the clinic. In stage 2, blood samples were collected on days 1, 7, and 14 just before (0 hour) and at 1, 2, 3, 4, and 10 hours after administration of the morning and evening doses of the study medication, as well as on days 2 to 6 and days 8 to 13 just before the morning and evening doses of the study medication, on day 15 at 24 hours after the last dose, on day 16 at 48 hours after the last dose, and on day 17 before discharge from the clinic. All blood samples were collected into tubes containing potassium ethylenediamine triacetic acid (K$_3$EDTA) anticoagulant and were centrifuged. Urine for measurement of PTC124 concentrations was collected from 0 to 4, 4 to 8, 8 to 12, and 12 to 24 hours after each administration of study medication (single-dose study) or from 0 to 4, 4 to 8, 8 to 12, and 12 to 24 hours after the morning dose on the first and last days of study drug administration (multiple-dose study). The urine was collected into 500-mL plastic containers containing no preservative. Both the plasma and urine samples were frozen at –20°C until shipment for analysis.

PTC124 concentrations in plasma or urine were analyzed using a validated high-performance liquid chromatography with tandem mass spectrometry (HPLC/MS/MS) method. PTC124 and its internal standard (3-[5-(2,6-difluorophenyl)-[1,2,4]oxadiazol-3-yl]-benzoic acid) were recovered by liquid-liquid extraction from K$_3$EDTA human plasma or human urine. The mixture was vortexed and centrifuged, and the organic layer was transferred to a clean glass culture tube. After evaporating the organic layer under nitrogen in a 50°C water bath, the residue was reconstituted, and 10 µL of the extract was injected on an HPLC system and quantified using a tandem mass spectrometer. The transition ions m/z 285 → 123 and m/z 303 → 141 were monitored for PTC124 and the internal standard, respectively.

For the assay of PTC124 in plasma, 3 sets of calibration standards were prepared, with ranges of 1 to 500 ng/mL, 0.1 to 10 µg/mL, and 0.5 to 200 µg/mL. For the assay of PTC124 in urine, 2 sets of calibration standards were prepared, with ranges of 1 to 500 ng/mL and 0.5 to 200 µg/mL. Quality control (QC) samples were prepared at 3 PTC124 concentration levels for each calibration range. Each analytical batch contained 1 set of calibration standards, a sample with internal standard, a plasma blank, duplicate QC samples at each of 3 concentration levels, and the study samples. The peak areas for PTC124 were determined using a PerkinElmer Series liquid chromatograph with a PE Sciex Series 3000 mass spectrometer for quantitation. Calibration curves were obtained by performing a linear regression (weighted 1/x²) on the calibration standards, using an Analyst v. 1.3.1 data reduction software package. The overall method precision (% coefficient of variation) for QC samples in the assay of PTC124 in plasma ranged from 0.42% to 6.59%, and the overall accuracy (% relative error [RE]) ranged from –3.65% to 7.97%. The precision of calibration standards ranged from 0.86% to 3.30%, and the %RE ranged from –4.10% to 2.85%. The mean correlation coefficient (r) for the analytical batches was >0.999. The overall method precision for QC samples in the assay of PTC124 in urine ranged from 2.29% to 8.77%, and the overall accuracy ranged from –2.35% to 3.99%. The precision of calibration standards ranged from 0.45% to 3.50%, and the accuracy ranged from –3.42% to 3.30%. The mean r for the analytical batches was >0.999.

**Data Analysis**

Analyses of subject demography and disposition, drug administration, safety parameters, drug palatability, and immunoblot results were primarily descriptive. Pharmacokinetic parameters were estimated from plasma concentration data using standard noncompartmental methods. To provide a more uniform method of assessment across all subjects, the mean noncompartmental half life (t$_{1/2}$) values were calculated from least squares linear regressions of log-transformed individual subject concentration-time data using all available time points, from 6 hours through 72 hours in the single-dose study and from 4 hours through 12 hours in the multiple-dose study. Dose proportionality of pharmacokinetic parameters and potential sex effects were examined by means of analysis of variance (ANOVA). In the food effect comparison, the ratio of the geometric means of the fed and fasted populations and the 90% confidence intervals for AUC and C$_{max}$ were computed from log transformed data. In addition, data for each subject were fitted to a 1-compartment model using a weighted nonlinear regression. All pharmacokinetic and statistical analyses were performed using WinNonlin (Version 4.0) and SAS (Version 8.2).

**RESULTS**

**Subject Demography and Disposition**

Sixty-two subjects participated in the 2 studies combined. The subjects included 33 men and 29 women who ranged in age from 18 to 30 years. Body weights...
and BMI values ranged from 46.4 to 93.2 kg and from 19.8 to 30.4 kg/m², respectively. The majority of subjects identified themselves as either Hispanic (76%) or African American (19%).

Eighteen subjects (10 men, 8 women) were enrolled in stage 1 of the single-dose study. Doses were escalated through the first 5 planned dose levels of 3, 10, 30, 100, and 200 mg/kg. Because adverse events occurred in all subjects at the 200-mg/kg dose level, the panel of subjects that was to receive the planned highest dose level of 300 mg/kg was instead administered drug at 150 mg/kg. At all dose levels, at least 6 subjects took active PTC124, as specified in the protocol. Because too few qualified subjects were available when the 3- and 150-mg/kg doses were administered, 1 subject and no subjects, respectively, took placebo at those dose levels. Four male subjects discontinued prematurely, of whom 2 were replaced. Of the 4 subjects, 2 subjects were discontinued because of laboratory abnormalities, 1 because he was lost to follow-up, and 1 because of a positive urine drug screen. All 18 enrolled subjects were included in the safety and pharmacokinetic evaluation. Protein elongation studies were performed on pooled samples from 6 subjects following administration of PTC124 at 200 mg/kg and from the 2 subjects receiving placebo at that dose level. Specific readthrough was tested in the in vitro luciferase reporter assay using plasma from these same 6 subjects. In stage 2, 12 subjects (6 men, 6 women) receiving a PTC124 dose of 50 mg/kg completed both sequences of the crossover design and were included in the food effect evaluation. A seventh male subject who was enrolled to replace 1 of the 6 men was unable to return for the second dosing sequence (and the male subject who was to have been replaced completed both sequences). Therefore, 13 subjects were included in the safety evaluation, and 12 subjects were included in the food effect evaluation.

As planned, in the multiple-dose study, different cohorts of 6 subjects (3 men, 3 women) took PTC124 at each of the 4 planned dose levels (10-50 mg/kg/dose bid) for 7 days, and an additional 6 subjects (3 men, 3 women) took 50 mg/kg/dose bid for 14 days. These 30 subjects were included in all safety and pharmacokinetic evaluations. In the second stage, 1 additional male subject received a single dose of study drug at 50 mg/kg and then withdrew from the study because of a family emergency. His limited data are included in the analyses of safety parameters but not of pharmacokinetic parameters. Protein elongation studies were performed on pooled samples obtained from 6 subjects prior to dosing on day 1 (untreated control) and on the same 6 subjects before and after day 14 administration of PTC124 at 50 mg/kg/dose bid.

Clinical Safety and Palatability

Symptomatic adverse events that occurred in the 2 studies were predominantly mild in intensity, none was considered serious, and none resulted in discontinuation from the study.

During the dose escalation stage of the single-dose study, no discernable pattern of drug-related symptomatic adverse events was observed at doses of 3, 10, and 30 mg/kg. One symptomatic drug-related adverse event (nausea) was reported by 1 of the 6 subjects who took PTC124 at a dose of 100 mg/kg. At the 150- and 200-mg/kg dose levels, most subjects experienced at least 1 event of headache, dizziness, nausea, vomiting, diarrhea, and/or abdominal pain; these adverse events were considered drug related. During stage 2, drug-related vomiting and headache were reported by 1 subject each during both sequences of the crossover (fed vs fasting) at the PTC124 dose of 50 mg/kg. Many of the events occurred coincident with the t_max for PTC124 and resolved spontaneously within 15 minutes to 8 hours after onset.

During the dose escalation stage of the multiple-dose study, adverse events of flatulence and abdominal pain were noted on day 3 of dosing by 2 subjects receiving PTC124 10 mg/kg/dose bid. These symptoms resolved spontaneously and did not recur despite continued dosing. Mild headache was noted as an adverse event on day 7 by 2 subjects receiving 30 mg/kg/dose bid. No drug-related adverse events were reported among any other subjects, including the 6 subjects dosed for 14 days with PTC124 at 50 mg/kg/dose bid during stage 2.

In the single-dose study, 1 female subject receiving PTC124 at the 200-mg/kg dose level experienced asymptomatic, reversible elevations in alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Abnormalities occurred between 6 and 16 days after dosing, rising to peak values of 661 U/L for ALT and 226 U/L for AST on day 14 (12.2- and 5.7-fold over the respective upper limits of normal of 54 U/L and 40 U/L); these changes were considered to be potentially drug related.

In the multiple-dose study, 2 of 6 subjects dosed at 20 mg/kg/dose bid, 3 of 6 subjects dosed at 30 mg/kg/dose bid, and 5 of 13 subjects dosed at 50 mg/kg/dose bid experienced low-grade, asymptomatic elevations in serum transaminase values, primarily ALT and AST. Values first exceeded the normal range between 2 and 14 days from the start of treatment.
The highest values were 102 IU for ALT and 65 IU for AST (1.9- and 1.6-fold over the respective upper limits of normal of 54 U/L and 40 U/L). These changes did not require cessation of PTC124 administration and normalized shortly after completion of PTC124 dosing. No notable concomitant abnormalities of serum alkaline phosphatase, gamma-glutamyl transferase (GGT), lactate dehydrogenase (LDH), or bilirubin were observed. No evidence of hepatic synthetic dysfunction (eg, abnormal coagulation values) was observed.

In the single-dose study, 1 male subject who received PTC124 3 mg/kg and 1 male subject who received PTC124 100 mg/kg were discontinued because of elevations in serum creatine kinase (CK) to values of 28 631 and 3036 U/L, respectively (upper limit of normal of 235 U/L). In both cases, the abnormalities were observed at 13 days after dosing, when the subjects were being rescreened for the next dosing period, and then resolved over several days. Another male subject who received 50 mg/kg in the crossover portion of the study started with an elevated serum CK value of 344 U/L and had episodic minimal elevations to as high as 304 U/L at outpatient follow-up visits. In the multiple-dose study, 1 subject who received 20 mg/kg/dose bid had normal CK values throughout the dosing period and then had an elevated level (1257 U/L) at the day 8 visit that decreased to within normal limits at follow-up 9 days later. Creatine kinase changes in these subjects were not considered drug related.

No medically significant changes in coagulation or hematological parameters occurred during either study. No significant urinary abnormalities or changes in vital signs or ECGs were noted in any subject in either study.

In responding to the palatability questionnaire in the single-dose study, subjects noted that the texture of PTC124 was relatively smooth at doses of 3, 10, 30, 50, and 100 mg/kg. Despite the fact that the concentration did not change over the dose range tested, some subjects noted an objectionably gritty or chalky consistency at doses of 150 or 200 mg/kg. No specific taste (sweet, salty, sour, or bitter) or odor was associated with the drug, and no persistent aftertaste was described. No appreciable differences were observed between the results obtained in the fasted and fed states. Over the range of doses tested in the multiple-dose study (10-50 mg/kg/dose bid), the subjects reported no specific taste (sweet, salty, sour, or bitter) or odor in association with ingestion of the drug, and no persistent aftertaste was described. The end-of-treatment assessments (day 7 or day 14) were essentially the same as the day 1 assessments.

**Evaluation of Nonspecific Codon Readthrough by Immunoblotting**

To determine whether PTC124 causes nonspecific readthrough of normal stop codons, we examined pooled PMBC and plasma samples obtained during the single-dose study for the presence of elongated forms of CRP, β2 microglobulin, and cystatin C. These elongated products could only appear if their normal stop codons were read through. As shown in Figure 2, no elongated products for any of these proteins were detected by immunoblotting of samples from either untreated or PTC124-treated subjects (receiving PTC124 at 200 mg/kg). Evaluation of cystatin C with an antibody specific to the elongated protein confirmed the lack of any elongated protein product in subject samples. Collectively, these results indicate no evidence that PTC124 induced or enhanced readthrough of normal stop codons. Of note, pooled plasma samples obtained at 2 and 8 hours postdose from these same subjects demonstrated specific premature stop codon ribosomal readthrough of luciferase protein in our in vitro luciferase bioassay (data not shown).

In the multiple-dose study, immunoblots of pooled PBMC and plasma samples were probed with antibodies against CRP, β2 microglobulin, or cystatin C (Figure 3). No evidence of any readthrough product was observed in the band patterns for these proteins when the subjects were still untreated (at day 1, time 0) or after they had received 14 days of PTC124 treatment at 50 mg/kg/dose bid.

**Pharmacokinetics**

Following administration of single escalating doses of PTC124 (3-200 mg/kg), the time of peak PTC124 plasma concentration (t_{max}) generally occurred ~1 to ~3 hours after administration. PTC124 plasma concentrations declined steadily after reaching the maximum concentration (C_{max}), and the mean half-life (t_{1/2}; using a fixed interval from 6 through 72 hours) ranged from ~3 to ~6 hours, with t_{1/2} generally increasing as the dose increased (Table I, Figure 4). Increases in mean C_{max} and mean area under the concentration-time curve to 24 hours (AUC_{24h}) were somewhat greater than dose proportional as the dose increased from 3 to 150 mg/kg but were less than dose proportional in the transition from 150 to 200 mg/kg (Table I). Intersubject variability likely contributed to apparent
variations from dose proportionality. When tested by means of ANOVA with sex and dose in the model, no significant sex effects were observed for any pharmacokinetic parameter.

Ingestion of a high-fat, high-calorie meal just prior to administration of 50 mg/kg of PTC124 slowed drug absorption, shifting the mean $t_{\text{max}}$ from $\sim0.9$ to $\sim2.5$ hours while blunting and broadening the shape of the mean concentration-time curve around the $C_{\text{max}}$ (Table I, Figure 5). The mean $t_{1/2}$ of $\sim5$ hours was essentially unchanged. The ratios (90% confidence interval [CI]) of the geometric means with fed versus fasted drug administration were 0.95 (0.83 1.10) for $C_{\text{max}}$ and 1.35 (1.13 1.63) for AUC. The effect of food on PTC124 pharmacokinetics was comparable between the sexes.

In subjects receiving PTC124 for 7 days in the multiple-dose study, frequent sampling was performed after the morning dose on days 1 and 7 (Table II, Figure 6). Mean $t_{\text{max}}$ values were in the range of $\sim2$ to $\sim4$ hours, whereas mean $t_{1/2}$ values (using a fixed interval from 4-12 hours) ranged from $\sim2$ to $\sim6$ hours. Increases in $C_{\text{max}}$, $C_{12}$, and $\text{AUC}_{0-12}$ were generally proportional to PTC124 dose as the dose increased from 10 to 50 mg/kg/dose bid. Exposures at each dose level were similar on day 1.
and on day 7. In subjects receiving PTC124 for 14 days, frequent blood sampling was performed after both the morning and evening doses on days 1, 7, and 14 (Table II, Figure 7). Exposures after the morning dose in these subjects were comparable to those observed after the morning dose in subjects who had received 50 mg/kg/dose bid for 7 days. Within the variability of the values, the exposures were generally similar over time across day 1, day 7, and day 14. Diurnal variation was apparent; on days 1, 7, and 14, mean $C_{\text{max}}$ values were 5%, 17%, and 43% higher, respectively, and mean AUC$_{0-12}$ values were 25%, 39%, and 50% higher, respectively, following the evening dosing than following the morning dosing. No significant sex-related effects were observed for any pharmacokinetic parameter.
In both studies, the percentage of the total PTC124 dose excreted in the urine as the parent drug over 24 hours was low (<2%). No apparent sex-related effects on urinary excretion of PTC124 were observed.

**DISCUSSION**

PTC124 is a novel, small-molecule compound that represents the first drug specifically designed to

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**Table I**  Mean (SD) Pharmacokinetic Parameters for Plasma PTC124 in a Single-Dose Study

<table>
<thead>
<tr>
<th>Parameter, Units</th>
<th>3 mg/kg (6)</th>
<th>10 mg/kg (6)</th>
<th>30 mg/kg (6)</th>
<th>100 mg/kg (6)</th>
<th>150 mg/kg (6)</th>
<th>200 mg/kg (6)</th>
<th>50 mg/kg (12)</th>
<th>50 mg/kg (12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$t_{max}$, h</td>
<td>(1.0)</td>
<td>(0.3)</td>
<td>(0.2)</td>
<td>(1.0)</td>
<td>(1.3)</td>
<td>(0.8)</td>
<td>(0.2)</td>
<td>(1.9)</td>
</tr>
<tr>
<td>$C_{max}$, µg/mL</td>
<td>6 (0.5)</td>
<td>27 (6)</td>
<td>90 (22)</td>
<td>210 (75)</td>
<td>260 (33)</td>
<td>314 (29)</td>
<td>109 (28)</td>
<td>99 (24)</td>
</tr>
<tr>
<td>$AUC_{0-24}$, µg•h/mL</td>
<td>(6) (16)</td>
<td>(97) (1195)</td>
<td>(984) (374)</td>
<td>(210) (309)</td>
<td>(216) (32)</td>
<td>(684) (900)</td>
<td>(210) (900)</td>
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</tr>
<tr>
<td>$AUC_{0-72}$, µg•h/mL</td>
<td>27 (6)</td>
<td>110 (22)</td>
<td>477 (75)</td>
<td>2059 (33)</td>
<td>2788 (31)</td>
<td>2795 (29)</td>
<td>661 (29)</td>
<td>872 (24)</td>
</tr>
<tr>
<td>Dose-normalized $C_{max}$, µg/mL/mg/kg</td>
<td>(0.2)</td>
<td>(0.6)</td>
<td>(0.7)</td>
<td>(0.8)</td>
<td>(0.2)</td>
<td>(0.1)</td>
<td>(0.6)</td>
<td>(0.7)</td>
</tr>
<tr>
<td>Dose-normalized $AUC_{0-24}$, µg•h/mL/mg/kg</td>
<td>(2.0)</td>
<td>(1.6)</td>
<td>(3.2)</td>
<td>(12.0)</td>
<td>(6.6)</td>
<td>(1.9)</td>
<td>(4.2)</td>
<td>(6.2)</td>
</tr>
</tbody>
</table>

*PTC124 dose was administered within 30 minutes after a high-fat, high-calorie meal.

*b. The mean noncompartmental t1/2 values were calculated from least squares linear regressions of log-transformed individual subject data using all available time points from 6 hours through 72 hours.*
Table II  Mean (SD) Pharmacokinetic Parameters for Plasma PTC124 in Multiple-Dose Study

<table>
<thead>
<tr>
<th>Parameter, Units</th>
<th>Stage 1 PTC124—7 days bid Dosing(^a) (n)</th>
<th>Stage 2 PTC124—14 Days bid Dosing(^a)(n)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 mg/kg—AM (6)</td>
<td>20 mg/kg—AM (6)</td>
</tr>
<tr>
<td>(t_{\text{max}}, \text{h})</td>
<td>3.8 (1.2)</td>
<td>3.2 (1.0)</td>
</tr>
<tr>
<td>(t_{1/2}, \text{h})</td>
<td>2.4 (1.1)</td>
<td>2.5 (1.8)</td>
</tr>
<tr>
<td>(C_{\text{max}}, \mu g/mL)</td>
<td>16 (2)</td>
<td>13 (2)</td>
</tr>
<tr>
<td>(C_{12,d}, \mu g/mL)</td>
<td>2.7 (2.8)</td>
<td>3.0 (1.9)</td>
</tr>
<tr>
<td>(AUC_{0-12}, \mu g\cdot h/mL)</td>
<td>91 (9)</td>
<td>81 (9)</td>
</tr>
<tr>
<td>Dose-normalized (C_{\text{max}}, \mu g/mL/mg/kg)</td>
<td>1.6 (0.2)</td>
<td>1.3 (0.2)</td>
</tr>
<tr>
<td>Dose-normalized (C_{12,d}, \mu g/mL/mg/kg)</td>
<td>0.3 (0.2)</td>
<td>0.3 (0.2)</td>
</tr>
<tr>
<td>Dose-normalized (AUC_{0-12}, \mu g\cdot h/mL/mg/kg)</td>
<td>9.1 (0.9)</td>
<td>8.1 (0.9)</td>
</tr>
</tbody>
</table>

\(^a\) Doses were given twice per day (bid) at ~8:30 AM and ~8:30 PM within 30 minutes after a meal.

\(^b\) The mean noncompartmental \(t_{1/2}\) values were calculated from least squares linear regressions of log-transformed individual subject data using all available time points from 4 hours through 12 hours.

\(^c\) Excludes 1 subject with an extremely high calculated \(t_{1/2}\) (85.4 hours) that was inconsistent with the values in other subjects after the evening dose on day 7 and was inconsistent with the range of calculated \(t_{1/2}\) values (3.3-8.5 hours) in this subject after other doses on days 1, 7, and 14.

\(^d\) PTC124 plasma concentration at 12 hours after dose. For day 14, the \(C_{12}\) values were not available; hence, \(C_{10}\) values are shown.

\(^e\) For day 14, last time point for 1 subject was missing due to early discharge from the clinic; therefore, \(AUC_{0-12}\) has been calculated using data from 5 subjects.
induce ribosomes to selectively bypass premature stop codons in mRNA during translation. Treatment with PTC124 is intended to induce restoration of full-length, functional protein production in patients with inherited disorders resulting from a nonsense mutation in a critical gene. We performed these phase I single-dose and multiple-dose healthy volunteer trials as the first clinical studies of a development program aimed at systematically validating the concept of nonsense mutation suppression while seeking a therapeutic advance for patients with CF, DMD, and other genetic diseases.

Several design details of these studies merit comment. It was necessary to enroll adults to these first-in-man studies of PTC124; however, given the need for future development of PTC124 in children and adolescents with genetic disorders, we specifically elected to enroll subjects of ages 18 to 30 years to obtain safety and pharmacokinetic data in a youthful population. The planned dose range (3-300 mg/kg) in the single-dose study was within allometrically scaled no-observed-adverse-effect levels (NOAELs) from rats and dogs; the range was intentionally broad so as to span the range of nonsense suppression activity observed in the in vitro translational readthrough assays and to ensure a thorough understanding of symptomatic or laboratory events as a prelude to phase II testing in patients who often have disease-related symptoms and laboratory abnormalities. We considered food effect information and pharmacokinetic modeling of the single-dose data as the basis for our selection of bid dosing at mealtimes in the subsequent multiple-dose study. Our intent in the multiple-dose trial was to explore a dose range (10-50 mg/kg/dose bid) that might achieve and maintain the target plasma concentrations observed in preclinical studies and, based on the toxicities observed, develop an appropriate safety monitoring plan for later phase II trials. In both studies, we dosed PTC124 by milligrams of drug per kilogram of body weight because this method will be used in later phase II to III studies to accommodate a large range of patient sizes (from young children to young adults). We also employed a suspension formulation knowing that children often have difficulty with ingestion of tablets or capsules. In the multiple-dose study, milk was coadministered because it was anticipated that the drug might eventually be mixed with milk when taken by children. We systematically assessed palatability to determine whether any formulation adjustments would be necessary for taste masking to enhance phase II compliance. For both studies, safety parameters were generally standard given the lack of unusual target organ toxicities in the nonclinical safety profile of PTC124. However, a special assessment to address the concern that PTC124 might permit generalized protein elongation due to nonspecific normal stop codon readthrough was a novel component of these studies. One limitation of these studies may have been the enrollment of a preponderance of Hispanic and African American subjects. Although these ethnic backgrounds reflected the catchment area of the clinic, they diverge from the Caucasian ancestry most commonly observed in patients with CF and DMD and emphasize the need...
for appropriate safety monitoring and additional pharmacokinetic assessment in translating these phase I data into the phase II setting.

In the single-dose study, nausea, vomiting, diarrhea, headaches, and/or dizziness occurred in most subjects receiving single doses of 150 and 200 mg/kg. Given the need for chronic compliance with PTC124 dosing in children and adolescents with genetic disorders, the consistent and repeated occurrence of such events would likely prove dose and compliance limiting. Thus, we determined 100 mg/kg to be the maximum tolerated single dose. When observed, these events began coincident with PTC124 \( C_{\text{max}} \) values and resolved rapidly, suggesting a relationship to \( C_{\text{max}} \) plasma concentration. Consistent with these data, phase I multiple-dose study subjects receiving PTC124 at doses of 10 to 50 mg/kg/dose bid for up to 14 days had lower \( C_{\text{max}} \) values and showed no clinically significant asymptomatic adverse events at any dose tested. Based on specific questioning of the subjects, PTC124 seemed palatable; there was no significant taste or odor that might prove to be a concern for phase II testing in young patients.

We observed elevations of ALT and AST in 1 subject treated at 200 mg/kg in the single-dose study and in 9 of 24 subjects treated at 20 to 50 mg/kg/dose bid in the multiple-dose study. These changes were asymptomatic, did not require cessation of PTC124 administration, and normalized shortly after completion of PTC124 dosing. No abnormalities of alkaline phosphatase, GGT, bilirubin, or coagulation parameters were observed. The pattern suggests a hepatocellular reaction to the drug without evidence of cholestatic or functional impairment.\(^{14,15}\) In the single-dose study, such changes were observed only at the highest dose level. In the multiple-dose study, such changes were observed only at dose levels of \( \geq 20 \) mg/kg bid (\( \geq 40 \) mg/kg/d). Given the small number of subjects, attempts at correlating the incidence or magnitude of these transaminase elevations with dose level, duration of drug administration, sex, ethnic background, \( C_{\text{max}} \), or AUC were not revealing. As is often the case,\(^{16-18}\) these findings had not been clearly presaged by rat or dog toxicology studies of PTC124.\(^{13}\) Both rats and dogs receiving PTC124 had dose-dependent hepatocellular hypertrophy and hepatic enlargement without histopathologic evidence of hepatitis. At 28 days, rats showed minimal elevations of ALT, alkaline phosphatase, and bilirubin, but values were largely within expected ranges, and in a subsequent 26-week rat study, only alkaline phosphatase was slightly increased (PTC Therapeutics, data on file). Based on the results of the phase I studies, we have instituted close follow-up of serum transaminase levels in patients enrolled in phase II studies. Of note, unlike gentamicin, PTC124 showed no evidence of nephrotoxicity based on repeated evaluations of serum creatinine, blood urea nitrogen (BUN), and urinalyses.

We also observed that 3 male subjects in the single-dose study had asymptomatic elevations in serum CK values, usually documented upon return to the clinic 2 weeks after prior dosing. Lack of correlation with PTC124 dose and the timing of these events seemed most consistent with exercise-induced muscle injury,\(^{19-21}\) perhaps relating to occupational or sports-related activities. Illicit, between-dosing cocaine use was occasionally documented in our study population, suggesting cocaine-related rhabdomyolysis as an alternate possible contributor to these events.\(^{22-24}\) In the multiple-dose study, when subjects were confined to the clinic without exercise or access to drugs during the 7 to 14 days of PTC124 administration, elevations in serum CK were not observed.

Several hypothetical reasons explain why PTC124 induction of ribosomal readthrough is specific for premature stop codons,\(^{25,26}\) and we have confirmed such specificity for the drug in preclinical model systems.\(^{11}\) However, we also wished to evaluate in the clinic whether PTC124 could induce nonspecific readthrough at normal stop codons that might lead to generalized protein elongation. To address this issue, we analyzed several high-abundance proteins (CRP, \( \beta \)2 microglobulin, and cystatin C) in human blood. The mRNA for each of these proteins is known to have a second in-frame stop codon downstream in its 3'-untranslated region. Between the stop codon at the end of the protein coding region and the second stop codon, there is an intervening sequence of nucleotides that, theoretically, could code for an elongation of the protein. Therefore, if PTC124 were to have the capacity to induce nonspecific ribosomal readthrough, it would be expected that the protein would be increased in size by a defined amount and that the corresponding change in molecular weight would be detectable by immunoblotting. With these 3 proteins, each type of stop codon—UGA (CRP), UAA (\( \beta \)2 microglobulin), and UAG (cystatin C)—was represented.

At the highest single dose of 200 mg/kg and after 14 days of treatment with the highest repeated dose of 50 mg/kg/dose bid, despite intentional autoradiograph overexposure to enhance sensitivity, immunoblotting revealed no change in the band patterns with any of the 3 tested proteins that would indicate induction or enhancement of a nonspecific PTC124
effect on readthrough of normal stop codons. These data are consistent with similarly negative findings in protein elongation analyses of multiple tissues from 14-day rat and dog studies of PTC124.11 Although evaluation of specific ribosomal readthrough of an endogenous premature stop codon was not possible in these healthy volunteers, we were able to assay the plasma used in the immunoblotting experiments for activity in our nonsense mutation suppression luciferase reporter system.11 Despite the absence of nonspecific normal stop codon readthrough in vivo, the PTC124 present in the plasma of the subjects was able to induce specific premature stop codon readthrough in vitro, thus providing context to the negative normal stop codon readthrough results.

The pharmacokinetics of PTC124 in both studies indicated general dose proportionality for $C_{\text{max}}$ and AUC and no significant sex effects on any parameter. Alterations in the pharmacokinetic profile when PTC124 was taken after a meal were relatively minor, with no change in $C_{\text{max}}$ and an increase in AUC by 35%; the data indicated a modest delay in drug absorption that acted to maintain plasma concentrations. In the multiple-dose study, pharmacokinetic parameters were consistent with those observed in the phase I single-dose study. As expected given the relatively short PTC124 $t_{1/2}$ ($\leq 6$ hours), drug accumulation was not observed with multiple dosing, and there was no evidence of substantial autoinduction of drug metabolism. Of interest was the finding of diurnal variation in PTC124 plasma concentrations, with greater exposures (in particular, higher trough concentrations) occurring during the night than during the day. Diurnal variation in plasma concentrations of drugs can result from a number of factors, including circadian or sleep-related changes in gastrointestinal motility; cardiac output; hormone levels; renal, hepatic, or mesenteric blood flow; and tissue fluid shifts.27,28 Close examination of individual subject data revealed this to be a consistent pattern, but the degree of variability and the small sample size precluded a specific determination of how shifts in absorption or excretion might have contributed to the observed differences between daytime and nighttime profiles.

Collectively, the results indicate that trough plasma concentrations exceeding target values associated with activity in preclinical model systems can safely be achieved and maintained. Application of the 1-compartment model, accounting for the diurnal variation, suggests that 3 times per day (tid) dosing with meals—administering 2 smaller doses at 6-hour intervals during the day and a larger dose at a 12-hour interval overnight (eg, at 7:00 AM, 1:00 PM, and 7:00 PM)—might optimally sustain target plasma concentrations while minimizing total exposures. This administration plan capitalizes on the effect of food in maintaining PTC124 plasma concentrations and on the nocturnal increase in plasma exposures and is likely to fit well with daily patterns of living for patients. This strategy is being implemented in phase II studies. As shown in Figure 8, the lower dose regimen of PTC124 (given tid at doses of 4, 4, and 8 mg/kg) is projected to maintain the steady-state trough drug concentration at the $\geq 2$-$\mu\text{g/mL}$ level that was associated with detectable activity in preclinical models.11,13 The higher dose regimen of PTC124 (given tid at doses of 10, 10, and 20 mg/kg) is projected to maintain the steady-state trough drug concentration at the $\geq 10$-$\mu\text{g/mL}$ level that was associated with maximal preclinical activity. Pharmacokinetic monitoring in phase II will permit evaluation of actual results against these projections.

In summary, development of PTC124 constitutes a novel therapeutic approach to the treatment of genetic disorders, coupling identification of patients having a specific genetic defect with application of a small-molecule therapy that has the potential to correct the phenotypic expression of that genetic defect. These phase I studies in healthy volunteers have established an important understanding of PTC124 safety, palatability, specificity, and pharmacokinetics.

Figure 8. Simulated mean plasma concentrations of PTC124 by dose level in subjects receiving PTC124 tid at 7 AM, 1 PM, and 7 PM within 30 minutes after a meal. Simulations are based on a multiple-dose 1-compartment model derived from data obtained in fed subjects taking part in the multiple-dose study and take diurnal variation into account. Doses were chosen so that the projected trough concentrations would exceed the target values of 2 and 10 $\mu\text{g/mL}$ in the majority of patients.
as a foundation to the design and conduct of ongoing phase II studies in patients with nonsense mutation–mediated CF29,30 and DMD.31

We commend the volunteer subjects who committed their time and effort despite the uncertainties of testing a new medication. We are indebted to Lawrence Galitz, MD, Allan Xu, PhD, and the clinical staff who were involved in the conduct of this study. The technical expertise of Jin Zhuo, MD, and Yuki Tomizawa, BS, in the performance of the immunoblotting studies is very much appreciated. We would also like to extend our gratitude to Liz Miller, MS, of EAM Clinical Sciences, for her assistance in the drafting of the manuscript.

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