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Single- and Multiple-Dose Pharmacokinetics and Pharmacodynamics of PN-943, a Gastrointestinal-Restricted Oral Peptide Antagonist of $\alpha 4\beta 7$, in Healthy Volunteers

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Abstract

PN-943 is an orally stable, gastrointestinal-restricted peptide that binds specifically to α4ß7 integrin on leukocytes, blocking leukocyte trafficking to and activation in the gut, inhibiting colon inflammation and reducing signs and symptoms of active ulcerative colitis. Two pharmacokinetic/pharmacodynamic studies were conducted in healthy volunteers. Study 1 was a first-in-human study with 40 male subjects receiving PN-943, 100 to 1400 mg or placebo, as single doses and 57 male subjects receiving PN-943, 100 to 1000 mg or placebo, as multiple doses. Study 2 was a randomized, crossover study comparing multiple doses of 450-mg PN-943 twice daily as a liquid solution and as an immediate-release tablet in 10 subjects. No subjects discontinued due to treatment-emergent adverse events. Consistent with the gastrointestinal-restricted nature of the peptide, systemic exposure was minimal; there was an approximate dose-proportional increase in area under the plasma concentration–time curve. There was minimal accumulation with once-daily dosing and an absence of time-dependent changes in pharmacokinetics. Administration of PN-943 after a high-fat meal reduced peak plasma concentration and area under the plasma concentration–time curve. There was minimal (<0.1%) urinary excretion of intact drug, and there was a dose-related increase in fecal excretion of intact PN-943. Dose-dependent increases in blood receptor occupancy and reduction in blood receptor expression were observed, supporting target engagement. Twice-daily dosing resulted in sustained receptor occupancy with low plasma fluctuations (143%). PN-943 was generally well tolerated following single and multiple oral doses with low systemic exposure. Twice-daily dosing resulted in sustained pharmacokinetics and pharmacodynamics, supporting further investigation in efficacy studies.

Keywords

$\alpha_4\beta_7$ antagonist, gastrointestinal restricted, pharmacokinetics, pharmacodynamics, PN-943

Ulcerative colitis is a chronic inflammatory bowel disease with a remitting and relapsing course, characterized by bloody diarrhea, abdominal cramps, and fatigue.[1,2] The pathogenesis is thought to result from inappropriate immune response to gastrointestinal antigens and environmental triggers in genetically susceptible individuals.[3] The highest prevalence is reported in Europe (505 per 100 000 persons) and North America (249 per 100 000 persons).[4] Ulcerative colitis has a significant negative impact on patient quality of life and presents a high economic burden on health systems.

Inflammatory bowel disease has been managed with corticosteroids, 5-aminosalicylates, and immunosuppressants, and more recently with the use of biologics

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targeted against specific mediators of inflammation. Therapeutic options for the long-term maintenance of remission in ulcerative colitis are limited.[5] 5-Aminosalicylates such as sulfasalazine, olsalazine, balsalazide, and various forms of mesalamine are effective only in mild to moderate disease, whereas patients with severe disease may be started on biologics.[6] Several monoclonal antibodies against tumor necrosis factor (TNF)-α (eg, infliximab, adalimumab, golimumab, and certolizumab) are now available.[7–10] Agents targeted against other cytokines involved in the inflammatory response such as ustekinumab against interleukin (IL)-12/IL-23, and tofacitinib, a pan–Janus kinase (JAK) inhibitor, are now part of the therapeutic options available for inflammatory bowel disease, and several IL-23 and sphingosine-1-phosphate receptor modulators are also currently under clinical investigation.[11–13]

In spite of the wide array of therapeutic options, there are still limitations in the treatment of inflammatory bowel diseases, and the agents available are not without risk. TNF-α inhibitors are ineffective in approximately one-fifth to one-third of the patients, and 10% to 15% of treated patients who show an initial benefit may lose response every year.[6,14–16] Cutaneous reactions are also the most common adverse reactions with anti-TNF treatments.[17] This includes injection site reactions, cutaneous infections, immune-mediated complications such as psoriasis and lupus-like syndrome, and, rarely, skin cancers. Tofacitinib can increase the risk of infection and may increase the risk of thrombosis or thromboembolic events. There is increasing recognition that mitigation of the local inflammatory response may hold promise. Orally administered budesonide and 5-aminosalicylates are effective locally, and various other locally acting agents, including AMT-101, an oral biologic fusion protein of interleukin 10,[18] TD-1473, a JAK inhibitor,[19] GB004, a hydroxylase inhibitor,[20] and BT-11, a lanthionine synthetase inhibitor,[21] have shown promise or are undergoing clinical investigation for inflammatory bowel disease. Local delivery through oral administration may allow higher doses of drug to be delivered to the target site without increasing systemic side effects.

The $\alpha 4\beta 7$ integrin, present on the cell surface of circulating memory T and B lymphocytes, is primarily involved in the recruitment of leukocytes to the gastrointestinal mucosa and associated lymphoid tissues. The major ligand for $\alpha 4\beta 7$, mucosal addressin cell adhesion molecule, is selectively expressed on the endothelium of the gastrointestinal vasculature and is present in increased concentrations in inflamed tissues.

Vedolizumab is an intravenously administered humanized immunoglobulin G monoclonal antibody directed against $\alpha_4\beta_7$ that has been approved for the treatment of moderate to severe ulcerative colitis and Crohn disease in adult patients who are not responding to $\geq$1 conventional treatments, such as steroids, immunosuppressive agents, or TNF inhibitors.[22–24] Due to the inconvenience and potential systemic risks of injectable treatments, an oral, gastrointestinal-restricted therapeutic that selectively targets the $\alpha_4\beta_7$ integrin may provide a significant benefit to patients with ulcerative colitis. Results from a small clinical study with PTG-100, a first-generation $\alpha_4\beta_7$ integrin antagonist, have demonstrated dose-dependent mucosal healing suggesting that an oral, gut-restricted, $\alpha_4\beta_7$ integrin antagonist may be effective in ulcerative colitis.[25] PN-943 is an orally stable peptide and a structural analog of PTG-100 that binds specifically to the $\alpha_4\beta_7$ integrin on leukocytes with a higher in vitro potency than PTG-100.[26] Preclinical studies have shown that PN-943 has minimal systemic absorption (<1%) in animals and is more effective than PTG-100 as measured by greater levels of target engagement and effects on T-cell trafficking. In a trinitrobenzenesulfonic acid–induced colitis rat model, PN-943 dosing caused a significantly lower mean colon histopathology score compared to rats treated with vehicle or PTG-100.[26] Through the blockade of leukocyte trafficking in the gut and local lymphocyte activation,[27] PN-943 may inhibit colon inflammation, reducing the signs and symptoms of ulcerative colitis. The present study investigated the safety, tolerability, pharmacokinetics, and pharmacodynamics of oral PN-943 in healthy male subjects.

Methods

Study Design

Two studies were conducted at a single clinical center (Nucleus Network, Melbourne, Australia). Study 1 was a 3-part first-in-human study in healthy male volunteers to assess the safety, tolerability, pharmacokinetics, and pharmacodynamics of a liquid solution formulation of PN-943. Part 1 was a randomized, placebo-controlled, double-blind study of single ascending doses of PN-943 in 40 male subjects divided into 4 equal cohorts. Dose escalation proceeded from 100 mg to 300 mg, 1000 mg, and 1400 mg. Subjects in the 300-mg dose cohort received treatment in the fasted state on 1 occasion and following a high-fat meal on a second occasion in a crossover fashion. The high-fat meal consisted of 2 eggs fried in butter, 2 strips of bacon, 2 slices of toast with butter, 4 ounces of hash brown potatoes, and 240 mL of whole milk. During part 1, subjects refrained from food and drink except water for 10 hours before and for 4 hours after dosing with the exception of subjects in the 300-mg dose cohort during the fed treatment. Part 2 was a randomized, placebo-controlled, double-blind multiple-ascending-dose study in 50 male subjects divided equally into 5 cohorts. Subjects received

once-daily dosing of PN-943 or placebo for 14 days. Doses evaluated in part 2 included 100 mg, 300 mg, and 1000 mg. During part 2, two cohorts of subjects (100 mg and 300 mg) received food approximately 30 minutes before each dose and another 2 cohorts of subjects (300 mg and 1000 mg) refrained from food for 10 hours before and for 1 hour after dosing. An additional cohort of 9 subjects in part 2 received 300 mg of PN-943 in a crossover fashion to evaluate the effect of meal timing on the pharmacokinetics and pharmacodynamics of PN-943. Subjects in this cohort received a meal 30, 60, or 90 minutes after PN-943 dosing. Part 3 was an open-label, randomized, crossover multiple-dose comparison of 900-mg once-daily and 450-mg twice-daily dosing of PN-943 as a liquid solution for 5 days. Subjects in part 3 refrained from food for 10 hours before and for 1 hour after dosing of PN-943.

The second study was a 5-day multiple-dose pharmacokinetic and pharmacodynamic study comparing the liquid formulation and a tablet formulation administered as 450 mg PN-943 twice daily in healthy male and female subjects. Subjects held food for 10 hours before and for 1 hour after the morning dose and for 1 hour before and after the evening dose of each day.

Both studies were conducted at a single clinical center, and the study protocols, subject information, and informed consent form were reviewed and approved by independent human research ethics committees (Alfred Health Human Research Ethics Committee, Melbourne VIC 3004, Australia for study 1; and Bellberry Human Research Ethics Committee, Eastwood South Australia 5063, Australia for study 2). The studies were conducted in accordance with the Declaration of Helsinki on biomedical research involving human subjects and International Conference on Harmonization Good Clinical Practice guidelines, and all study procedures were conducted by scientifically and medically qualified personnel. Written informed consent explaining the nature, purpose, and potential risks and benefits of the study was provided by subjects before they participated in any study-related activities.

Study Subjects

Both studies used similar procedures for screening and enrollment. Subjects were screened within 21 days of enrollment. Eligible subjects were aged 18 to 55 years inclusive with a body mass index between 18 and 30 kg/m^2, who were in good general health, with no significant medical history or clinically significant abnormalities on physical examination. The first-in-human study (study 1) enrolled only male subjects, while the study evaluating the tablet formulation (study 2) enrolled men and women who agreed to use highly effective methods of contraception based on the Clinical Trials Facilitation and Coordination Group for the duration of the study and for 90 days after the last dose.

Subjects were excluded if they had a history of clinically significant endocrine, gastrointestinal, cardiovascular, hematologic, hepatic, immunologic, renal, respiratory, or genitourinary abnormalities or diseases, or had clinically significant laboratory abnormalities, including impaired renal function (serum creatinine >106 μmol/L or estimated creatinine clearance <80 mL/min) or alanine aminotransferase or aspartate aminotransferase values >1.2 times the upper limit of normal.

Procedures

Study 1. The single- and multiple-ascending-dose phase of the study consisted of sequential dose escalations in 10 subject per dose cohort. Participants were randomized to receive PN-943 or matching placebo as a 60-mL oral solution in a ratio of 8:2. Blood samples for pharmacokinetics were collected before dosing and for 48 hours after dosing following single doses. In the multiple-ascending-dose phase, blood samples were obtained on days 1 to 3 and 14 to 16; on day 8, samples were obtained before dosing and at 4 and 12 hours after dosing. On day 10 of the multiple-ascending-dose phase, subjects were required to collect all urine for the 0- to 6-, 6- to 12-, 12- to 18-, and 18- to 24-hour intervals after dosing, and on day 11, subjects were required to collect all fecal samples for 24 hours. The decision to proceed to the next dose level was made by the investigator and the safety monitoring committee based on acceptable safety and tolerability of the lower dose.

Study 2. This study was a randomized, open-label, 2-treatment, 2-period, multiple-dose study to determine the safety, tolerability, pharmacokinetics, and pharmacodynamics of an immediate-release (IR) tablet and a liquid solution of PN-943. The study allowed comparison of a solid dose formulation to the liquid formulation that had been investigated in the first-in-human study. Subjects received 450 mg of PN-943 twice daily for 5 days as one 300-mg and one 150-mg dosage strength IR tablet administered every 12 hours and 450 mg of PN-943 twice daily for 5 days as a liquid solution administered every 12 hours in a randomized fashion.

Stability studies were conducted for the dosing solutions over the anticipated concentration range (1-25 mg/mL) and demonstrated that dosing solutions were stable for up to 3 months when stored at 2 to 8°C. Dose solutions were formulated in 50 mM of phosphate buffer, pH 7.4, and were prepared weekly by a qualified pharmacist.

Dose Selection Rationale

The starting dose in the first-in-human single- and multiple-dose study was based on consideration of the no-observed-effect level from 28-day toxicology

studies in rats and cynomolgus monkeys, and the receptor occupancy noted in cynomolgus monkeys. The no-observed-effect level determined in rats and monkeys translated to a human equivalent dose of approximately 145 mg using standard allometric scaling and a 10-fold safety margin. A starting dose of 100 mg was selected, with initial stepwise escalations of approximately 3-fold.

The dose selected for study 2, comparing a tablet and the oral solution formulation, was based on the pharmacokinetic and pharmacodynamic profile from part 3 of the first-in-human study and the anticipated dose planned in an efficacy study in patients with moderate to severe ulcerative colitis.

Sample Analysis Method for PN-943 in Humans

Concentrations of PN-943 in plasma, urine, and fecal samples from study 1 and plasma and fecal samples from study 2 were assayed using a validated high-performance liquid chromatography–tandem mass spectrometry method.

PN-943 and its stable isotope-labeled internal standard were isolated from plasma using a protein precipitation procedure using acetonitrile : methanol : formic acid solution (74.9% : 25.0% : 0.1%). PN-943 and its stable-labeled internal standard were isolated from urine stabilized with 10% Triton X-100 (100 μL/10 mL urine) using a dilution procedure with aqueous solution of 75% acetonitrile. Feces samples were homogenized with ethanol and phosphate buffer solution (50% : 50%). PN-943 and its stable isotope-labeled internal standard was extracted from feces homogenate using a solid-phase extraction procedure with 5% ammonia solution in ultra-pure water and eluted with methanol/trifluoroacetic acid mixture (99% : 1%). Analysis of PN-943 in all matrices was conducted using a Zorbax 300 StableBond C18 column (2.1 × 150 mm) with 5-μm particle size (Agilent Technologies, Santa Clara, California) and a C18 guard cartridge (4.0 × 21 mm; Phenomenex, Torrance, California). Mobile phase A comprised 5% acetonitrile, 94.9% water, and 0.1% formic acid; and mobile phase B comprised 94.9% acetonitrile, 5% water, and 0.1% formic acid. The samples were analyzed using a liquid chromatography coupled with tandem mass spectrometry using an API4000 detector (AB SCIEX, Framingham, Massachusetts) using a turbospray ion source with positive multiple reaction monitoring mode with *m*/*z* for Q1 mass set to 918.0.

Calibration curves for PN-943 in human plasma were linear from 0.200 to 100 ng/mL using 200 μL of plasma with correlation coefficients ≥0.9889. Calibration curves for PN-943 in urine were linear from 20.0 to 10,000 ng/mL using 50 μL of urine with correlation coefficients ≥0.9881. Calibration curves for PN-943 in fecal homogenate were linear from 0.100 to 50.0 μg/mL using 50 μL of fecal homogenate with correlation coefficients ≥0.9926. The interday (between-day) precision (% coefficient of variation) ranged from 7.2% to 13.2% for plasma, 4.1% to 18.0% for urine, and 4.3% to 8.2% for fecal homogenate. Intraday (within-day) precision (% coefficient of variation) ranged from 1.3% to 15.8% for plasma, 1.7% to 13.5% for urine, and 2.2% to 8.7% for fecal homogenate. The interday accuracy (% bias) ranged from –4.7% to 0.7% for plasma, –6.8% to 7.2% for urine, and –5.6% to 4.0% for fecal homogenate. Intraday accuracy ranged from –19.5% to 4.9% for plasma, –19.5% to 20.0% for urine, and –7.7% to 6.0% for fecal homogenate.

Study End Points

The objective of this first-in-human study was the safety and tolerability assessments following single and multiple dosing with PN-943. In addition, the study characterized the single- and multiple-dose pharmacokinetics and pharmacodynamics of PN-943, and evaluated the effect of a high-fat meal on PN-943 pharmacokinetics, and compared twice-daily and once-daily dosing. Safety assessments, adverse events, and laboratory assessments are summarized descriptively for the placebo and each PN-943 dose.

The end points for the second study comparing the oral solution and the tablet formulation were pharmacokinetics and pharmacodynamics.

Pharmacokinetic Analyses

Pharmacokinetic parameters were estimated by noncompartmental methods (Phoenix WinNonlin; Certara, Princeton, New Jersey). Peak plasma concentration (C_{max}) and time to peak plasma concentration were observed values. The elimination rate was estimated from the slope of the least squares regression on the terminal log-linear phase. Area under the plasma concentration–time curve from time 0 to the last quantifiable concentration (AUC_t) was estimated by a linear trapezoidal method and was extrapolated to infinity (AUC_{inf}) by dividing the last quantifiable concentration by the elimination rate. For calculation of plasma concentration summary statistics, values below the limit of quantification were set to 0. Steady-state fluctuation in the plasma concentration was calculated as $\frac{C_{max}-C_{min}}{C_{average}}$. Accumulation was estimated as the ratio of the parameter (C_{max} and AUC_{inf}) following the last dose of the multiple-dose regimen to the value on day 1 ($AUC_{inf\ Day\ 14\ or\ Day\ 5}/AUC_{inf\ Day\ 1}$ and $C_{max\ Day\ 14\ or\ Day\ 5}/C_{max\ Day\ 1}$). Bioavailability of the IR tablets was estimated relative to the liquid solution using the ratio of the AUC_{inf} for the 2 treatments.

Pharmacodynamic Assays for α4β7 Receptor Occupancy and Receptor Expression

Translational biomarkers such as receptor occupancy have been validated as pharmacodynamic markers through use in preclinical studies and in clinical trials with vedolizumab binding against the $\alpha_4\beta_7$ receptor.[28,29] In this study, a flow cytometry–based assay was designed to quantify the amount of $\alpha_4\beta_7$ integrin on the cell surface that is occupied by PN-943 or the amount of $\alpha_4\beta_7$ expression on the cell surface of circulating lymphocytes in response to engagement by PN-943. Similar approaches for measuring receptor occupancy and receptor expression using flow cytometry have been used previously.[30–33] Briefly, in this assay, each heparinized whole blood sample is first treated with saturating amount of an unlabeled competing peptide serving as the "blocked" control for 100% receptor occupancy, or no peptide, serving as the "unblocked" sample to measure the level of blocking by orally administered PN-943. After incubation, the blood is stained with a subsaturating concentration of Alexa647-labeled peptide, followed by staining with the cell surface marker panel (CD45, CD3, CD4, CD45RA, CD19, immunoglobulin D, and the anti-$\alpha_4\beta_7$ antibody vedolizumab). After staining is completed, the samples are treated with a red blood cell lysis and fixation buffer, washed and acquired on a flow cytometer. To quantify receptor occupancy on $\alpha_4\beta_7$ expressing memory CD4 T cells, the median fluorescence intensity (MFI) of Alexa647-labeled peptide within the vedolizumab + memory CD4+ T cells was used. Receptor occupancy was calculated according to the following formula: [Percent RO] = (1 – ([Unblocked] – [Blocked]) / ([Baseline Unblocked] – [Baseline Blocked])) × 100.

Expression of α4β7 is defined by MFI of vedolizumab within the memory CD4+ T cells from the unblocked samples. Receptor expression was calculated as percent change of MFI from baseline for the vedolizumab stain.

Pharmacokinetic/Pharmacodynamic Analysis

The in vivo PN-943 plasma concentration-receptor occupancy relationship was characterized using a sigmoid E_{max} (Hill) model, $RO = E_0 + \frac{E_{max} - E_0}{1 + (\frac{EC_{50}}{C})^{\gamma}}$ using Prism version 9.0.0 (GraphPad Software, San Diego, California).

Statistical Analyses

No formal sample size estimations were performed. Eight subjects received oral PN-943, and 2 subjects received placebo in each dose cohort in the single- and multiple-ascending-dose study. Ten subjects were enrolled in the second study comparing the immediate-release tablet formulation to the oral solution. The enrollment in each study was considered adequate to assess the tolerability and safety and to allow characterization of the pharmacokinetics and pharmacodynamics of PN-943.

Results

Subject Characteristics and Disposition

A total of 97 healthy male subjects were enrolled in study 1 with 40 subjects enrolled in the single-dose phase and 57 subjects in the multiple-dose phase. An overview of the disposition of subjects is presented in Figure S1. All subjects completed their dosing with PN-943 or placebo as planned. Two subjects withdrew consent for personal reasons unrelated to safety; 1 subject did not want to remain in the clinical unit, and a second subject was uncomfortable with the venous cannula. Subject characteristics are summarized in Table S1. The average age was 28.7 years in the single-dose phase and 30.9 years in the multiple-dose phase.

Ten subjects were enrolled in study 2, and 9 subjects completed both treatments. One subject discontinued the study on day 1 following the oral solution treatment due to an adverse event of acute tonsillitis that was considered unrelated to the study drug. Subject characteristics for study 2 are presented in Table S1.

Safety and Tolerability

An overview of the treatment-emergent adverse events (TEAEs) following single doses of PN-943 (study 1) is presented in Table S2. A total of 23 TEAEs were reported by 14 subjects during the single-ascending-dose phase. Of the 14 subjects who experienced TEAEs, 12 received PN-943 (21 events) and 2 received placebo (2 events). Of the 21 TEAEs following PN-943, 7 were considered related to treatment; both TEAEs following placebo were considered treatment related. Incidence of TEAEs did not demonstrate a systematic dose relationship. All TEAEs were mild or moderate except for a severe headache in a subject treated with 100 mg of PN-943 that was not considered related to treatment. All subjects recovered from the adverse events (AEs) and no subjects were withdrawn due to AEs. Nervous system disorders were the most frequently reported TEAEs. AEs reported in ≥2 subjects included nausea, upper respiratory tract infections, headache, presyncope, and somnolence. No clinically relevant changes were observed in respiratory rate or vital signs, clinical laboratory parameters (hematology, coagulation, serum chemistry, or urinalysis), or in the interpretation of electrocardiograms or QTc interval.

An overview of the TEAEs following multiple doses of PN-943 is shown in Table S3. Thirty subjects in the group receiving multiple doses of PN-943 reported a total of 68 AEs. All but 2 occurrences were mild in

Table 1. Single-Dose Pharmacokinetics of PN-943 Following Oral Dosing (Mean ± SD)

	100 mg (N = 8)	300 mg Fasted (N = 8)	300 mg High Fat Meal (N = 8)	1000 mg (N = 8)	1400 mg (N = 8)
C_{max}, ng/mL	2.11 ± 1.15	6.55 ± 3.38	1.58 ± 0.71	15.3 ± 4.11	23.5 ± 19.0
t_{max}, h[a]	2.0 (1.0, 4.0)	3.0 (1.0, 8.0)	4.0 (2.0, 4.0)	4.0 (0.5, 4.0)	4.0 (1.0, 12.0)
AUC_t, ng • h/mL	12.9 ± 7.27	44.3 ± 21.5	11.5 ± 4.62	138 ± 33.7	257 ± 173
AUC_{inf}, ng • h/mL	16.5 ± 8.70[b]	57.6 ± 23.6[b]	–[c]	151 ± 31.7[d]	260 ± 173
$t_{1/2}$, h	3.05 ± 0.71[b]	4.02 ± 1.37[b]	–[c]	5.26 ± 0.91[d]	5.74 ± 1.35

AUC_t, area under the plasma concentration–time curve to the last observed concentration; AUC_{inf}, area under the plasma concentration–time curve extrapolated to infinity; C_{max}, maximum observed plasma concentration; SD, standard deviation; t_{max}, time to maximum concentration; $t_{1/2}$, half-life.
[a] Median (min, max)
[b] N = 4.
[c] Not reported due to insufficient data.
[d] N = 7.

severity. One report of upper respiratory tract infection was characterized as moderate, and 1 report of influenza that occurred after release from the clinical unit was categorized as severe and considered a serious AE. Four subjects receiving placebo reported a total of 6 mild TEAEs, primarily gastrointestinal disorders. TEAEs reported in ≥2 subjects in the multiple-ascending-dose phase included abdominal discomfort, flatulence, upper respiratory tract infection, back pain, dizziness, and headache. Nervous system disorders, particularly headache, were the most commonly reported TEAEs. No clinically relevant changes were observed in respiratory rate, vital signs, or clinical laboratory parameters or on the electrocardiograms.

Of the 10 subjects enrolled in study 2 comparing dosing with IR tablets to an oral solution, 9 subjects completed both treatments. One subject experienced an AE of moderate tonsillitis unrelated to the treatment that led to discontinuation from the study. The incidence of TEAEs was similar across both treatments. The most common AE was headache, with all other AEs being reported in only 1 subject.

Pharmacokinetics

The mean plasma concentration-time profiles following single doses of PN-943 are presented in Figure 1A. The single-dose pharmacokinetics of PN-943 are summarized in Table 1. Median time to peak plasma concentration was 2 to 4 hours. C_{max} increased from 2.11 ng/mL to 23.5 ng/mL, and AUC_{inf} increased from 16.5 ng • h/mL to 260 ng • h/mL as PN-943 doses increased from 100 mg to 1400 mg. There was a dose-proportional increase in AUC_{inf} and a slightly less than dose-proportional increase in C_{max} over the dose range of 100 mg to 1400 mg PN-943. The mean elimination half-life at the lower doses (100 and 300 mg) was 3.1 to 4.0 hours and at higher doses (1000 and 1400 mg) was 5.3 to 5.7 hours.

The mean plasma concentration-time profiles following multiple doses of PN-943 are presented in Figure 2A. The pharmacokinetics of PN-943 following multiple doses is summarized in Table 2. There was an approximate dose-proportional increase in C_{max} and in AUC_{inf} for the 100- and 300-mg dose groups in the fed condition on day 14 and between the 300-mg and 1000-mg dose groups in the fasted condition on day 14. Median time to peak plasma concentration ranged from 2 to 4 hours. The mean elimination half-life was 5.2 to 7.7 hours. Consistent with the half-life, a comparison of the C_{max} and the AUC_t values for individual subjects on day 1 and on day 14 at 300 mg and 1000 mg suggested minimal (≤30%) accumulation with once-daily dosing. Comparison of the AUC_{inf} values on day 1 and AUC_t values on day 14 indicated the absence of time-dependent changes in the pharmacokinetics of PN-943.

During the multiple-ascending-dose phase, 24-hour collection of urine and feces was undertaken in the 300-mg and 1000-mg dose groups. Only a small fraction of PN-943 was recovered intact in urine over 24 hours, with recoveries of 0.028%, 0.056%, and 0.056% in the 300-mg fasted, 300-mg fed, and 1000-mg dose groups, respectively. There was a dose-related increase in the 24-hour fecal recovery of PN-94 with 0.73%, 1.78%, and 16.8% PN-943 recovered intact in the 300-mg fasted, 300-mg fed, and 1000-mg dose groups, respectively.

Effect of Food

The effect of a high-fat meal on the pharmacokinetics of PN-943 was evaluated in a crossover fashion at 300 mg during the single-ascending-dose portion of study 1. Administration of PN-943 within 30 minutes of consuming a high-fat meal reduced the peak concentration and exposure compared to the fasted state (Table 1). Mean PN-943 peak plasma concentrations were 6.55 ng/mL in the fasted state and 1.58 ng/mL in the fed state. Median time to peak concentration was delayed by 1 hour following a high-fat meal.

Table 2. Multiple-Dose Pharmacokinetics of PN-943 Following Oral Dosing (Mean ± SD)

	100 mg Fed (N = 8)		300 mg Fed (N = 8)		300 mg Fasted (N = 8)		1000 mg Fasted (N = 8)	
	Day 1	Day 14	Day 1	Day 14	Day 1	Day 14	Day 1	Day 14
C_{max}, ng/mL	0.745 ± 0.409	0.898 ± 0.454	2.32 ± 1.34	2.80 ± 1.66	7.23 ± 4.29	4.72 ± 1.85	13.8 ± 3.86	17.1 ± 6.05
t_{max}, h[a]	4.0 (2.0, 8.0)	4.0 (2.0, 4.0)	4.0 (1.0, 4.0)	4.0 (4.0, 8.0)	2.0 (0.5, 2.0)	2.0 (1.0, 4.0)	2.0 (0.25, 4.0)	2.0 (1.0, 4.0)
AUC_t, ng • h/mL	4.58 ± 3.12	5.87 ± 3.38	16.4 ± 10.8	21.0 ± 9.99	42.9 ± 17.9	39.5 ± 11.5	141 ± 43.6	184 ± 81.8
AUC_{inf}, ng • h/mL	9.81[b]	8.33[b]	39.7[b]	–	46.3 ± 19.2	43.9 ± 13.0[d]	157 ± 55.7	207 ± 125
$t_{1/2}$, h	3.85[b]	7.40[b]	7.96[b]	–[c]	5.16 ± 1.92	7.31 ± 3.86[d]	6.60 ± 1.99	7.70 ± 3.69

AUC_t, area under the plasma concentration–time curve to the last observed concentration; AUC_{inf}, area under the plasma concentration–time curve extrapolated to infinity; C_{max}, maximum observed plasma concentration; SD, standard deviation; t_{max}, time to maximum concentration; $t_{1/2}$, half-life.

[a] Median (min, max).

[b] N = 1.

[c] Not reported due to insufficient data.

[d] N = 7.

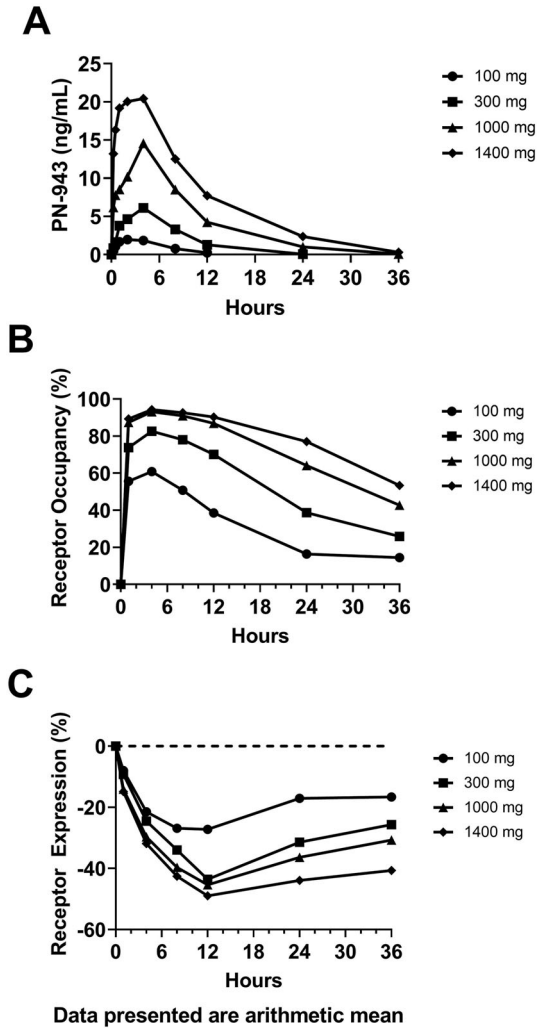

Figure 1. Single dose pharmacokinetics (A) and pharmacodynamics of PN-943 based on receptor occupancy (B) and receptor expression (C).

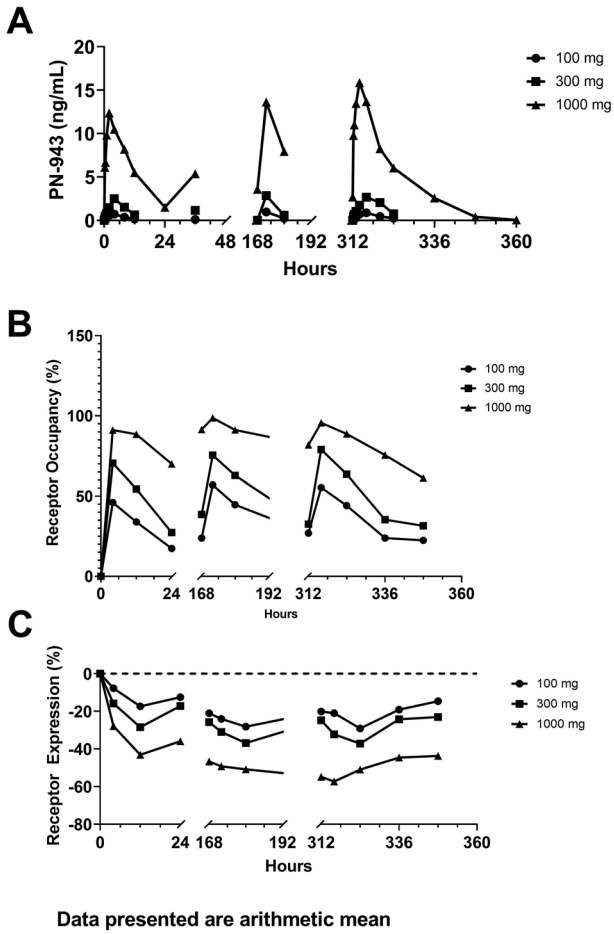

Figure 2. Multiple-dose pharmacokinetics (A) and pharmacodynamics of PN-943 based on receptor occupancy (B) and receptor expression (C).

The effect of the interval between PN-943 dosing and consumption of a meal was examined in study 1. Subjects received a meal 30, 60, or 90 minutes after a single dose of 300-mg PN-943. The median time-to-peak PN-943 plasma concentrations was 1, 2, and 4 hours for the 30-, 60-, and 90-minute treatment groups. There was a small increase in the C_{max} and AUC_t values when food was delayed 60 or 90 minutes compared to 30 minutes following PN-943, with minor differences noted between the 60- and 90-minute delay. Based on the more favorable C_{max} and AUC_t values noted for the 60-minute delay in food compared to the 30-minute delay, dosing for additional cohorts in the multiple ascending dose incorporated a 1-hour fasting interval before and after dosing of PN-943.

Table 2 presents a comparison of the pharmacokinetics of 300 mg of PN-943 following a meal compared to refraining from a meal within 1 hour of dosing PN-943 as part of the multiple-ascending-dose phase of study 1. The median time-to-peak concentration was 4 hours when PN-943 was administered following a meal, whereas it was 2 hours when food was withheld for 1 hour following PN-943. Peak plasma concentrations were lower when PN-943 was administered shortly after a meal compared to when food was administered 1 hour following dosing (day 1 C_{max} were 7.23 ng/mL and 2.32 ng/mL for the fasted and fed 1 hour after dosing, respectively).

Pharmacokinetics of Once-Daily and Twice-Daily Dosing

The effect of dosing regimen was evaluated following 900 mg once daily for 5 days and 450 mg twice daily for 5 days in a randomized crossover fashion in part 3 of study 1. A summary of the pharmacokinetic comparison of once-daily and twice-daily dosing is presented in Table S4. Peak concentrations were noted at a median of 2 hours for both dosing regimens on day 1 and on day 5. The steady-state peak concentrations were

Table 3. Steady-State Pharmacokinetics of PN-943 Following Oral Dosing of 450 mg Twice-Daily as a Liquid Solution and as an Immediate-Release Tablet (Mean ± SD)

	Liquid Solution 450 mg Twice Daily (N = 9)	Immediate Release Tablet 450 mg Twice Daily (N = 9)
C_{max}, ng/mL	9.36 ± 4.81	7.67 ± 2.97
t_{max}, h[a]	2.0 (1.0, 8.0)	2.0 (2.0, 4.0)
AUC_{0-24}, ng • h/mL	106 ± 34.7	86.3 ± 30.9
AUC_{0-12}, ng • h/mL	65.6 ± 38.6	53.8 ± 20.9
AUC_{12-24}, ng • h/mL	39.3 ± 15.2	32.4 ± 11.4
Bioavailability,[b] %	–	85.3 ± 36.2
Accumulation[c] C_{max}	1.25 ± 0.46	2.19 ± 1.0
Accumulation[c] AUC	1.36 ± 0.33	1.57 ± 0.43
C_{trough}, ng/mL	1.98 ± 0.83	1.86 ± 0.97

AUC_{0-24}, area under the plasma concentration–time curve to 24 hours; AUC_{0-12}, area under the curve to 12 hours; AUC_{12-24}, area under the plasma concentration–time curve from 12 to 24 hours; C_{max}, maximum observed plasma concentration; C_{trough}, trough concentration; SD, standard deviation; t_{max}, time to maximum concentration; $t_{1/2}$, half-life.
[a] Median (min, max).
[b] Bioavailability relative to liquid solution.
[c] Ratio of C_{max} (and AUC) on day 5 relative to day 1.

14.2 ng/mL for once-daily dosing and 9.96 ng/mL for twice-daily dosing. Dose-adjusted AUC over the dosing interval was comparable for the 2 treatment regimens. Consistent with the half-life of PN-943, there was minimal accumulation with once-daily dosing, and the accumulation was approximately 1.6- to 1.7-fold with twice-daily dosing. Twice-daily dosing of 450 mg of PN-943 as a liquid solution resulted in sustained plasma concentrations as reflected by the lower peak-to-trough fluctuation (143% vs 245%) and higher trough concentrations (3.25 ng/mL vs 1.78 ng/mL) compared to 900 mg once daily (Table S4).

Pharmacokinetics of a Liquid Solution and an IR Tablet Formulation of PN-943

The steady-state pharmacokinetics of an IR tablet of PN-943 administered as 450 mg twice daily for 5 days compared to the liquid solution used in the first-in-human study is summarized in Table 3. Figure 3A presents the mean steady-state plasma concentration–time profile for the 2 formulations. Both formulations had a similar median time to peak concentration (2 hours), while the peak concentration was ≈20% lower for the IR tablet compared to the liquid solution. The IR tablet formulation had a bioavailability of ≈85% relative to the liquid solution. Twice-daily dosing of the tablet formulation resulted in an accumulation of ≈2.2-fold based on C_{max} and 1.6-fold based on AUC. Steady-state trough concentrations of PN-943 were comparable for the IR tablet and the liquid solution (1.86 ng/mL and 1.98 ng/mL, respectively).

Pharmacodynamics

The mean pharmacodynamics of $\alpha_4\beta_7{}^+$ memory $CD4^+$ T cell as measured by mean percent receptor occupancy and mean receptor expression following single doses of PN-943 is summarized in Table 4. The time course of the mean percent receptor occupancy and mean receptor expression following single doses of PN-943 is presented in Figure 1B and 1C. The mean time to peak $\alpha_4\beta_7$ memory $CD4^+$ T-cell receptor occupancy was ≈4 hours. Mean peak receptor occupancy increased in a dose-related manner, ranging from 61.8% at 100 mg

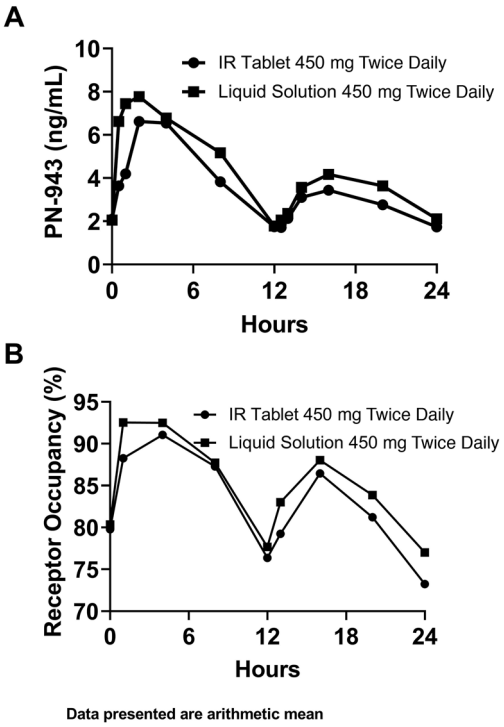

Figure 3. Steady-state pharmacokinetics (A) and pharmacodynamics (B) following 450-mg twice-daily dosing of PN-943 as a liquid solution and as immediate-release (IR) tablets.

Table 4. Single-Dose Pharmacodynamics of PN-943 Following Oral Dosing (Mean ± SD)

	Placebo (N = 8)	100 mg (N = 8)	300 mg (N = 8)	1000 mg (N = 8)	1400 mg (N = 8)
Receptor Occupancy					
RO_{max}, %	8.6 ± 7.0	61.8 ± 10.8	83.4 ± 7.92	93.6 ± 2.04	94.8 ± 3.55
t_{max} for RO, h	12 ± 11	3.8 ± 2.2	4.6 ± 2.3	4.1 ± 1.9	4.6 ± 3.2
Average RO,[a] %	1.7 ± 4.9	38.9 ± 12.5	64.3 ± 6.6	81.0 ± 3.5	86.0 ± 6.8
Receptor Expression					
RE_{max}, %	–5.06 ± 11.7	–28.2 ± 7.97	–43.6 ± 3.69	–45.4 ± 3.70	–49.0 ± 8.20
t_{max} for RE, h	18 ± 9.1	11 ± 6.0	12 ± 0	12 ± 0	12 ± 0
Average RE,[b] %	–1.7 ± 4.9	–21.6 ± 7.1	–32.4 ± 3.0	–36.3 ± 2.9	–40.3 ± 6.9

RE_{max}, maximum receptor expression, RO_{max}, maximum receptor occupancy; SD, standard deviation; t_{max} for RO, time to maximum receptor occupancy; T_{max} for RE, time to maximum receptor expression
[a] Average receptor occupancy over 24 hours.
[b] Average receptor expression over 24 hours.

to 94.8% at 1400 mg. Peak receptor occupancy for the 1000-mg and 1400-mg dose cohorts was similar, indicating that receptor occupancy saturation was achieved by single doses of ≈1000 mg PN-943. Mean change in receptor expression increased with dose, ranging from −28.2% for 100 mg of PN-943 to –49.0% for the 1400-mg dose group.

The pharmacodynamics of PN-943 and measured by $\alpha_4\beta_7^+$ memory $CD4^+$ T-cell percent receptor occupancy and receptor expression following multiple doses of PN-943 is presented in Figure 2B and 2C. Table 5 summarizes the mean pharmacodynamics following multiple doses of PN-943. Mean peak memory T-cell receptor occupancy following multiple doses of PN-943 achieved a peak at ≈4 hours. Mean percent receptor occupancy on day 1 of the multiple-dose cohorts were comparable to the corresponding doses in the single-dose cohorts. On day 1, the mean peak receptor occupancy following 300 mg and 1000 mg was 77.8% and 91.3%, respectively. There was a small increase in the peak percent receptor occupancy with continued daily dosing over 14 days. On day 14, the mean peak receptor occupancy in following 300 mg and 1000 mg was 79.7% and 95.6%, respectively.

Administration of PN-943 within 30 minutes of consuming a high-fat meal reduced the pharmacodynamic effect, consistent with the effect of a high-fat meal on the pharmacokinetics. Peak percent receptor occupancy following 300 mg of PN-943 in the fasted state was 83.4% compared to 61.4% when PN-943 was administered within 30 minutes following a high-fat meal. Delaying food for 60 minutes following PN-943 administration improved the pharmacodynamic profile compared to consuming a meal within 30 minutes following dosing or dosing PN-943 following a high fat meal. There was relatively little difference in the steady-state (day 14) pharmacodynamic effects when PN-943 was taken in the fasted state or when food was administered 60 minutes following PN-943 dosing (Table 5).

The pharmacodynamic effect of 900 mg once daily and 450 mg twice daily was examined as part of the multiple-ascending-dose phase of study 1. A summary of the pharmacodynamic effect on receptor occupancy by dosing regimen is presented in Table S5. On day 1, the 900-mg once-daily regimen had a mean peak receptor occupancy of 94.5% compared to 86.5% for the 450 mg twice-daily regimen. While both treatment regimens resulted in a similar peak receptor occupancy on day 5 (94.9% and 91.9% for 900 mg once daily and 450 mg twice daily, respectively), the twice-daily regimen provided a more sustained pharmacodynamic effect. Notably, the area under the effect curve on day 5 was higher for the twice-daily regimen compared to the once-daily regimen. The average receptor occupancy based on the 24-hour area under the effect curve on day 5 was 85.3% for the twice-daily regimen and 79.2% for the once-daily regimen. The twice-daily regimen also provided a sustained effect as noted by the minimal difference in the peak and trough receptor occupancy. In addition, the intersubject variability in receptor occupancy at trough for the 450-mg twice-daily treatment was 11.3% to 15.2% on day 5 compared to 26.3% to 33.6% for the 900-mg once-daily treatment, suggesting a more consistent effect for the twice-daily regimen.

The steady-state $CD4^+$ $\alpha4\beta7$ memory T-cell percent receptor occupancy pharmacodynamics following twice-daily PN-943 as the IR tablet or as the liquid solution is presented in Figure 3B. The steady-state receptor occupancy pharmacodynamics following the liquid solution and IR tablet is summarized in Table 6. Peak receptor occupancy was noted at 3 to 4 hours for both formulations. Mean steady-state peak receptor occupancy for the IR tablet was 91.9% with an average 24-hour receptor occupancy of 83.6% compared to a peak receptor occupancy of 93.8% and an average 24-hour receptor occupancy of 85.7% for the liquid solution.

Table 5. Multiple-Dose Pharmacodynamics of PN-943 Following Oral Dosing (Mean ± SD)

	Placebo (N = 8)		100 mg Fed (N = 8)		300 mg Fed (N = 8)		300 mg Fasted (N = 8)		1000 mg Fasted (N = 8)	
	Day 1	Day 14	Day 1	Day 14	Day 1	Day 14	Day 1	Day 14	Day 1	Day 14
Receptor Occupancy										
RO_{max}, %	10.6 ± 15.0	16.3 ± 21.2	46.8 ± 11.2	55.5 ± 8.82	70.4 ± 7.39	78.9 ± 5.89	77.8 ± 5.13	79.7 ± 5.40	91.3 ± 2.93	95.6 ± 0.97
t_{max} for RO, h	9.5 ± 4.8	21 ± 17	6.0 ± 3.7	5.0 ± 2.8	4.0 ± 0	4.0 ± 0	4.0 ± 0	4.0 ± 0	7.0 ± 4.1	4.0 ± 0
Average RO,[a] %	5.0 ±1 3.0	8.0 ± 15.8	30.0 ± 7.7	40.5 ± 7.5	47.2 ± 7.5	57.8 ± 3.9	53.9 ± 6.5	61.1 ± 6.0	77.1 ± 3.7	81.5 ± 15.5
Receptor Expression										
RE_{max} (%)	–0.85 ± 12.7	–9.8 ± 19.8	–17.4 ± 7.5	–29.1 ± 7.2	–28.4 ± 4.4	–37.2 ± 4.7	–33.1 ± 5.2	–41.5 ± 5.9	–43.1 ± 4.0	–57.3 ± 2.9
t_{max} for RE, h	12.0 ± 8.3	9.5 ± 8.0	14.0 ± 4.2	12.0 ± 0	12.0 ± 0	12.0 ± 0	12.0 ± 0	6.0 ± 3.7	12.0 ± 0	4.0 ± 0
Average RE,[b] %	0.06 ± 7.8	–5.9 ± 11.0	–12.3 ± 6.6	–23.8 ± 6.9	–20.1 ± 3.4	–31.7 ± 4.0	–25.1 ± 4.4	–37.7 ± 5.2	–33.9 ± 3.4	–51.3 ± 3.1

RE_{max}, maximum receptor expression, RO_{max}, maximum receptor occupancy; SD, standard deviation; t_{max} for RE, time to maximum receptor expression; t_{max} for RO, time to maximum receptor occupancy.
[a] Average receptor occupancy over 24 hours.
[b] Average receptor expression over 24 hours.

Table 6. Steady-State Pharmacodynamics of PN-943 Following Oral Dosing of 450 mg Twice-Daily as a Liquid Solution and as an Immediate-Release Tablet (Mean ± SD)

	Liquid Solution 450 mg Twice Daily (N = 9)	IR Tablet 450 mg Twice Daily (N = 9)
RO_{max}, %	93.8 ± 2.82	91.9 ± 4.56
t_{max} for RO, h	3.1 ± 2.4	3.8 ± 2.0
Average $RO_{0\text{-}24}$, %/h	85.7 ± 4.3	83.6 ± 5.5
Average $RO_{0\text{-}12}$, %/h	89.7 ± 5.6	86.4 ± 5.2
Average $RO_{12\text{-}24}$, %/h	83.5 ± 6.6	80.9 ± 6.0

$RO_{0\text{-}24}$, receptor occupancy over 24 hours; $RO_{0\text{-}12}$, receptor occupancy from 0 to 12 hours; $RO_{12\text{-}24}$, receptor occupancy from 12 to 24 hours; RO_{max}, maximum receptor occupancy; SD, standard deviation; t_{max} for RO, time to maximum receptor occupancy.

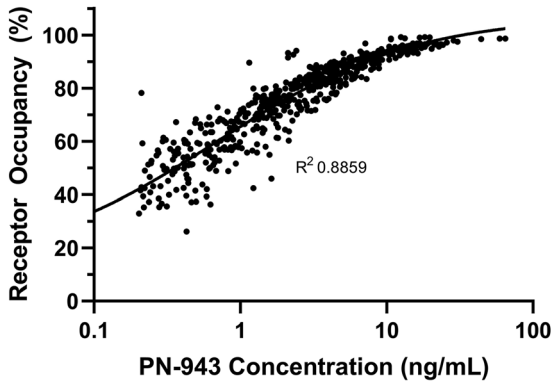

Figure 4. PN-943 plasma concentration–receptor occupancy relationship.

Table 7. Concentration-Receptor Occupancy Relationship Following Oral Administration of PN-943

	Estimate (95% Confidence Interval)
E_0, %	12.5 (10.4-14.0)
E_{max}, %	107.2 (100.8-117.3)
EC_{50}, ng/mL	0.69 (0.55-0.95)
EC_{80}, ng/mL	5.9 (3.5-13.1)
γ	0.65 (0.52-0.77)

Parameters for a sigmoid Hill relationship for receptor occupancy: E_0, baseline occupancy; E_{max}, maximum occupancy; EC_{50}, PN-943 plasma concentration resulting in 50% receptor occupancy; EC_{80}, PN-943 plasma concentration resulting in 80% receptor occupancy; γ, Hill coefficient.

Pharmacokinetic-Pharmacodynamic Correlation

The in vivo PN-943 plasma concentration-receptor occupancy relationship was characterized using a sigmoid E_{max} (Hill) model (Figure 4). The estimated parameters are presented in Table 7. The baseline (E_0) was 12.5%, and the E_{max} was 107.2%, with a span of 94.7%. The estimated PN-943 plasma concentration resulting in 50% receptor occupancy and 80% receptor occupancy were 0.69 ng/mL and 5.9 ng/mL, respectively.

Discussion

PN-943 is an oral gastrointestinal restricted peptide that binds specifically to the $\alpha4\beta7$ integrin on leukocytes that is being investigated as a potential oral therapy for patients with ulcerative colitis. The gastrointestinal-restricted nature of the peptide and enhanced gastrointestinal stability allow local effect and have the potential to enhance efficacy while minimizing the potential for AEs associated with systemic exposure. A similar approach of gastrointestinal restricted therapy has also been adopted for several other inflammatory bowel disease drug candidates such as TD-1473,[19] a JAK inhibitor; GB004,[20] a small-molecule hypoxia-inducible factor 1-α stabilizer; and BT-11,[21] a lanthionine synthetase C-like 2 agonist. Typically, gut-restricted agents are selected for their gut-targeted profile to limit systemic exposure and improve on-target effect and to achieve a greater exposure in the gastrointestinal tract relative to the plasma. PN-943 had plasma concentrations that were >6000-fold lower than the fecal concentrations. Systemic concentrations of PN-943 on a dose-adjusted basis were also much lower than those noted for other oral inflammatory bowel disease agents characterized as gastrointestinal or gut restricted.[19–21]

The objective of the current studies with PN-943 was to assess the safety and tolerability of PN-943 after single and multiple dosing. Additional objectives were to evaluate the pharmacokinetic and pharmacodynamic profile of PN-943 after single- and multiple-ascending-oral-dose administration; to assess the effects of food on the pharmacokinetics and pharmacokinetics; to compare once-daily and twice-daily dosing; and to describe the pharmacokinetics and pharmacodynamics of an immediate-release solid dosage form of PN-943.

PN-943 was well tolerated following single doses of up to 1400 mg and multiple doses of up to 1000 mg once daily for 14 days in the first-in-human study. TEAEs were all mild except for 1 report of severe headache following a single administration of the lowest dose of PN-943 (100 mg) and a report of influenza reported following 900 mg once daily. None of the TEAEs led to subject withdrawal from the study. TEAEs noted in ≥2 subject following repeated dosing included abdominal discomfort, flatulence, upper respiratory tract infection,

back pain, dizziness, and headache, with headaches being the most frequently reported TEAEs. Treatment with PN-943 did not result in any safety findings with regards to clinically meaningful changes in vital signs or clinical laboratory values, and no evidence of QTc prolongation was observed. There was no difference in the TEAE profile following dosing of PN-943 twice daily as an IR tablet or as a liquid solution.

Following single oral doses, PN-943 had a moderate rate of absorption, with maximum plasma concentrations noted at ≈4 hours. The increase in PN-943 AUC was approximately dose proportional, whereas the increase in C_{max} was slightly less than dose proportional. PN-943 demonstrated low systemic exposure following single and multiple dosing. The terminal half-life was 3.1 to 5.7 hours in the fasted state and 5.2 to 7.7 hours in the fed state. Consistent with the terminal half-life, when administered once daily and twice daily, the accumulation of PN-943 was ≈0.9- and 1.6-fold, respectively. There was an absence of time-dependent pharmacokinetics as evidenced by the similar AUC_{inf} on day 1 and AUC_t on day 14 (Table 2).

There was a dose-dependent increase in $\alpha_4\beta_7$ receptor occupancy following PN-943 administration, reaching a mean peak receptor occupancy >90% with doses of 900 mg. Trough receptor occupancy following once-daily dosing of 100-mg and 1000-mg PN-943 was ≈25.4% and 78.6%, respectively, and 79.2% following 450-mg PN-943 twice daily. These receptor occupancy data indicate that PN-943 concentrations remain at a sufficient level to allow once- or twice-daily dosing. Pharmacokinetic/pharacodynamic correlation showed concentration-dependent receptor occupancy with an asymptote at complete receptor occupancy and an estimated 50% inhibitory concentration of 0.69 ng/mL and an 80% inhibitory concentration of 5.9 ng/mL. The estimated 50% inhibitory concentration for receptor occupancy noted in humans (0.69 ng/mL) compares very favorably with the potency of PN-943 against memory CD4+ T cells expressing $\alpha4\beta7$ isolated from human peripheral blood mononuclear cells adhering to recombinant mucosal addressin cell adhesion molecule (0.73 ng/mL).

Systemic concentrations of PN-943 following oral administration were generally low, consistent with the intestinally restricted nature of the drug and the very low oral bioavailability (<1%) that has been noted in mice and cynomolgus monkeys.[26] There was a dose-dependent increase in fecal recovery of PN-943 following oral administrations, ranging from approximately 1% to 2% at 300 mg to 16.8% at 1000 mg. PN-943 is a small disulfide-containing cyclic peptide. Orally administered peptides encounter a harsh environment along the gastrointestinal tract, including pH conditions ranging from pH <2 in the stomach to pH 8 in the duodenum, as well as proteolytic enzymes such as gastric hydrolases (pepsins), pancreatic hydrolases (trypsin, chymotrypsin, elastase, aminopeptidases, and carboxypeptidase A and B), and intestinal brush-border membrane-bound enzymes (carboxypeptidases, endopeptidases, and aminopeptidases).[34] The highly acidic environment in the stomach results in degradation of peptide drugs through destabilization of the 3-dimensional structure.[35] PN-943 was chemically engineered to be stable under these conditions based on ex vivo assays using gastric and colonic extracts from mice, rats, and humans. Oral dosing in mice of a fluorescent dye conjugate of PTG-100, an analog of PN-943, and imaging by fluorescence microscopy or immunohistochemistry showed the peptide accumulates in the lamina propria of tissues from the small intestine.[36] Consistent with a locally acting drug, 30-mg/kg oral dosing of PTG-100 in mice demonstrated high exposure and occupancy of T-cell $\alpha4\beta7$ receptors in the gastrointestinal system compared to the blood.[27] Efficacy studies in a rat trinitrobenzenesulfonic acid colitis model have similarly shown that PN-943 causes significant improvements in local disease histopathology at comparable oral doses.[26] Therefore, it is likely that PN-943 also localizes within human gastrointestinal tissues at similarly high concentrations to exert its therapeutic effect despite its low systemic exposure.

In a comprehensive study with a diverse array of peptide drugs, Wang et al[37] showed that with the exception of [D-ser]-gonadorelin, smaller peptides were more stable in human, porcine, and simulated gastric fluid whereas large peptides, save for somatostatin, were rapidly degraded within 10 minutes. In human small intestinal fluids, small and large peptides degraded rapidly with the exception of cyclosporin and the disulfide bridge–containing peptides octreotide and desmopressin. The faster degradation of larger peptides in gastric fluid may be attributed to the presence of a higher number of pepsin-susceptible peptide bonds, high structural flexibility, a greater number of H bond acceptors/donors, and a higher polar surface area.[37–38] These authors also showed the importance of pepsin relative to pH in the degradation of peptides in the gastric environment. Pepsin specifically cleaves amino acids at L-Phe, L-Met, L-Leu, and L-Trp, adjacent to a hydrophobic amino acid.[39]

Peptide and protein stability in the gastrointestinal tract is an inherent problem associated with oral administration, whether for local action or for systemic delivery. Numerous studies have indicated that various factors such as amino acid sequence, molecular size, and exposure to the gastrointestinal environment, including pH and enzymatic action, play a key role in determining peptide stability and potential for oral absorption. Cyclization though sulfide bond linkage, and

N-methylation provide some resistance to enzymatic degradation and may also improve oral absorption.[40–41]

The presence of low detectable intact PN-943 concentrations in the plasma following oral administration indicates that PN-943 is able to traverse the gastrointestinal wall. In addition, ≈0.03% to 0.06% of the drug was detected intact in the urine. Orally administered peptides typically have a low oral bioavailability. While the systemic concentrations of PN-943 are low, they were sufficient to achieve and maintain >80% receptor occupancy at trough following once-daily or twice-daily dosing.

Administration of PN-943 within 30 minutes of a high-fat meal reduced the oral absorption of PN-943. While there was not a direct correlation between systemic exposure and fecal recovery, directionally, the data indicated that corresponding with the reduction in absorption following the high-fat meal, there was an increase in fecal recovery. It is not surprising that a reduction in oral absorption of PN-943 was noted in the presence of a high-fat meal. A similar observation of reduced absorption has been made with salmon calcitonin, a 32-amino-acid peptide hormone and with octreotide, a synthetic octapeptide analog of human somatostatin. Dosing of salmon calcitonin after meal intake resulted in a significant reduction in maximum concentration and AUC.[42] An oral octreotide capsule (20 mg) was administered to 24 participants in the fasting state and within 30 minutes of consuming a high-fat, high-calorie meal.[43] The high-fat meal had a pronounced effect, reducing octreotide C_{max} and AUC by ≈90%. Compounds that exhibit permeability-limited absorption are more prone to display a negative food effect.[44]

The steady-state pharmacokinetic and pharmacodynamic profile of the IR tablet formulation of PN-943 was generally similar to the liquid formulation used in the first-in-human study. Twice-daily dosing of 450-mg PN-943 as IR tablets resulted in sustained pharmacokinetics and an average receptor occupancy of ≈84%.

Conclusions

Following single and multiple ascending doses, PN-943 was safe and well tolerated when given orally to healthy subjects over a wide dose range. Consistent with a gastrointestinal-restricted peptide, PN-943 had low systemic exposure with a pharmacokinetic profile that supports once or twice daily dosing. Twice daily dosing of PN-943 resulted in a sustained receptor occupancy. The safety, tolerability, and pharmacokinetic/pharmacodynamic profile of PN-943 in healthy subjects supports the continued clinical evaluation of this novel gastrointestinal-restricted targeted treatment for inflammatory bowel diseases.

Conflicts of Interest

All authors are employees of Protagonist Therapeutics, Inc. and own stock in the company.

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