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Pharmacokinetics, pharmacodynamics, and tolerability of an aqueous formulation of rusfertide (PTG-300), a hepcidin mimetic, in healthy volunteers: A double-blind first-in-human study

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Abstract

Objectives: Rusfertide is a potent peptide mimetic of hepcidin being investigated for the treatment of polycythemia vera. This randomized, placebo-controlled, double-blind study evaluated the safety, pharmacokinetics, and pharmacodynamics of single and repeated subcutaneous doses of an aqueous formulation of rusfertide in healthy adult males.

Methods: Subjects received single doses of 1, 3, 10, 20, 40, or 80 mg rusfertide or placebo. A separate cohort of subjects received two doses of 40 mg rusfertide or placebo 1 week apart. Blood samples for pharmacokinetics and pharmacodynamics were collected, and adverse events, clinical laboratory tests, 12-lead electrocardiograms, and vital signs were monitored.

Results: Rusfertide was well tolerated. There were no serious or severe treatment-emergent adverse events, and no patterns of clinically important adverse events, or laboratory, vital sign, or electrocardiogram abnormalities. Mean maximum rusfertide plasma concentration (C_{max}) and area under the concentration–time curve increased with dose, but less than dose proportionally. Median time to C_{max} was 2–4.5 h for 40 and 80 mg rusfertide and 8–24 h for lower doses. Apparent clearance and half-life increased with dose. Single doses of rusfertide 1–80 mg were associated with dose-dependent decreases in serum iron and transferrin-iron saturation.

Conclusions: Rusfertide was well tolerated and showed dose-dependent pharmacokinetics and pharmacodynamics.

KEYWORDS
hepcidin mimetic, pharmacodynamics, pharmacokinetics, rusfertide, tolerability

1 | INTRODUCTION

Hepcidin is a 25-amino acid peptide hormone produced primarily by the liver[1,2]; it is the key regulator of iron homeostasis and is itself regulated by systemic iron and inflammation. Hepcidin controls systemic iron flux by modulating the levels of ferroportin on the cell surface of enterocytes in the duodenum (regulating iron absorption), hepatocytes (to transport stored iron), and macrophages (to transfer

recycled iron), which control the levels of systemic iron.[3–6] To date, ferroportin is the only cellular exporter of unbound iron in vertebrates.[7,8] Hepcidin binds to the extracellular domain of ferroportin, thereby inducing internalization and lysosomal degradation of ferroportin and preventing cellular egress of iron.[9] Hepcidin also occludes the central cavity that exports iron in ferroportin.[10] A common feature of diseases such as polycythemia vera (PV) and hereditary hemochromatosis is hepcidin deficiency.[5,11–13] Production of hepcidin is increased in response to high systemic iron, and this results in degradation of ferroportin, decreased iron release from enterocytes, and iron storage in macrophages.[4] Hepcidin production declines when systemic iron levels are low; this leads to increased ferroportin expression and restoration of iron levels. Hepcidin is generated at a high rate and is quickly cleared by the kidneys; it has a half-life of several minutes.[4,14]

PV is a myeloproliferative disorder affecting more than 100 000 people in the United States[15] that is marked by a somatic driver mutation in the janus-activated kinase 2 gene (JAK2). The vast majority (~95%) of PV patients exhibit the acquired JAK2V617F mutation and approximately 3% have acquired mutations in exon 12 of the JAK2 gene.[16] Phenotypic manifestations of PV include erythrocytosis, bone marrow erythroid and megakaryocytic hyperplasia, aquagenic pruritis, fatigue, microvascular symptoms, and symptomatic splenomegaly.[17] Uncontrolled erythrocytosis in PV leads to hyperviscosity due to increased red cell mass and increased risk of pulmonary hypertension and thrombosis. Patients with PV have a heightened risk of thrombotic events, cardiovascular mortality, and burdensome symptoms such as fatigue, problems with concentration, and aquagenic pruritus.[18–21] The incidence of major thrombosis following PV diagnosis is approximately 2.70 per 100 patient-years.[19,22,23] A population-based study found that hazard ratios for thrombosis in patients with PV compared with controls at 3 months, 1 year, and 5 years were 4.2, 2.5, and 1.8, respectively.[24] Patients with PV who have hematocrit levels >45% have a higher risk of thrombotic complications and cardiovascular death than those who have hematocrit levels <45%.[22] Patients aged ≤60 years and with no previous vascular events are categorized as low risk and generally treated with phlebotomy and low-dose aspirin; however, the risk of vascular events remains high for these patients. Indeed, only 20%–30% of low-risk patients treated with phlebotomy are able to maintain hematocrits <45%.[25,26] Those aged >60 years, or those with prior thrombotic events, are categorized as high risk and typically receive phlebotomy with concurrent cytoreductive drugs (e.g., hydroxyurea, ruxolitinib, interferon alpha). While phlebotomy is integral to the treatment of PV, it may lead to iron deficiency, increased symptom burden, and lost productivity when used frequently or long term.[27]

The PV erythroid phenotype has been directly linked to hepcidin expression; endogenous hepcidin upregulation alleviates erythroid disease, whereas hepcidin ablation worsens it.[28] PV mice expressing the orthologous JAK2 mutation have shown significantly reduced splenomegaly along with normalized hematocrit levels following the administration of minihepcidin peptides.[29] In a mouse PV model with JAK2-V617F mutations, administration of a hepcidin mimetic peptide sequestered iron into macrophage storage compartments, restricted iron availability for erythropoiesis, and lowered hematocrit.[30]

Hemochromatosis is the result of insufficient hepcidin production or, in exceptional circumstances, hepcidin resistance.[31,32] Significantly lower prohepcidin concentrations have been reported in hemochromatosis patients compared with hemodialysis patients with renal anemia and to healthy controls.[33] Correspondingly, significantly lower hepcidin concentrations have been seen in homozygous hemochromatosis patients compared with healthy controls.[34]

Rusfertide (also referred to as PTG-300) is a synthetic peptide mimetic of hepcidin being developed as a potential therapy for PV and hemochromatosis in which manipulation of iron availability may be effective. It acts in a similar way as hepcidin——it inhibits ferroportin expression on the cell surface——with a half-maximal effective concentration of 5 nM.[35] In vitro studies examining the internalization of ferroportin suggest that rusfertide has 6- to 11-fold higher potency than hepcidin.[35,36] Studies in patients with PV indicate that rusfertide rapidly and robustly reduces hematocrit <45%, essentially eliminating the need for phlebotomies, and improving PV-related symptoms.[37–39] In an open-label study, rusfertide treatment rapidly reduced serum iron concentrations and transferrin-iron saturation (TSAT) reduced the rate of phlebotomy and limited liver iron accumulation in patients with hemochromatosis.[40]

Here, we present the results of the first-in-human trial of rusfertide. This study investigated the safety, tolerability, pharmacokinetics (PK), and pharmacodynamics (PD) of an aqueous formulation of rusfertide in healthy subjects, with the aim of identifying a dosing regimen suitable for the assessment of clinical efficacy.

2 | METHODS

2.1 | Participants and procedures

Eligible participants were nonsmoking, healthy male adults aged 18–60 years. They had a body mass index of 18–30 kg/m^2 and were in good general health, with clinical laboratory values in the normal range (or any deviations considered not clinically significant) and no significant medical history. Exclusion criteria included any history of iron deficiency, iron overload, and liver or renal disease according to the subject's medical history and medical records.

2.2 | Study design and treatment

This was a randomized, double-blind, placebo-controlled, phase 1 study of ascending doses of rusfertide in healthy male subjects. It was conducted at a single clinical center in Australia in accordance with the principles of the Declaration of Helsinki (2000) and the International Conference on Harmonization Good Clinical Practice Guideline. Institutional human research ethics committee approval and written informed consent from all subjects were obtained prior to any evaluations or study procedures. The study was registered

with the Australian New Zealand Clinical Trials Registry (ACTRN12617000453381).

In the first part of the study, escalating single doses of subcutaneous rusfertide (1, 3, 10, 20, 40, or 80 mg) were administered to groups of men randomized to rusfertide ($n = 8$ each for doses of 1–40 mg; $n = 5$ for 80 mg), or matching placebo ($n = 2$ for doses of 1–40 mg; $n = 1$ for 80 mg). Following the single-dose phase, an additional group of subjects received two 40 mg once-weekly subcutaneous doses of rusfertide ($n = 5$) or placebo ($n = 1$). Subcutaneous injections were administered to the lower quadrants of the abdomen. Dosing in the single-dose portion was initiated with a sentinel group of two subjects, with one subject receiving rusfertide and the other receiving placebo; the remaining subjects in the group were dosed 48 h later. Safety and tolerability data for 7 days after dosing were reviewed for each group of subjects prior to any dose escalation; available PK and PD data were also reviewed prior to any dose escalation. In the second part of the study, a single group of five subjects received two subcutaneous doses of 40 mg rusfertide or matching placebo, given 1 week apart. The follow-up period for all subjects was ≥4 weeks.

Subjects fasted overnight for ≥10 h prior to planned dosing, and they continued to fast for 2 h after dosing. No food, or drink containing grapefruit juice, could be consumed within 24 h prior to initial dosing and throughout the study period. Caffeinated drinks were prohibited during confinement in the clinical unit. Subjects abstained from alcohol from 24 h before study drug administration to discharge from the clinical unit; at all other times, alcohol was allowed, but was limited to no more than 3 units per day. Smoking was not permitted during the study, and subjects were instructed to avoid unaccustomed, strenuous physical activity from Day −1 until discharge from the clinic. Rusfertide was formulated in 0.9% sodium chloride and supplied in prefilled 1-mL syringes. Placebo doses were provided similarly in a prefilled syringe, without any active ingredient.

2.3 | Study endpoints

The primary objective of this first-in-human study was to characterize the safety and tolerability of rusfertide following single doses. Additional objectives involved characterizing the PK and PD of subcutaneous rusfertide.

2.4 | Tolerability and safety assessments

Safety assessments were performed during screening, at admission to the clinical unit, before dosing, and at scheduled intervals after dosing. Assessments included clinical laboratory tests (hematology, coagulation, and clinical chemistry), physical examinations, vital signs, and 12-lead electrocardiograms (ECGs). Adverse events (AEs) were also monitored, and treatment-emergent adverse events (TEAEs) were coded using the Medical Dictionary for Regulatory Activities version 20.0. Tolerability assessments incorporated physical examinations, ECG assessments, laboratory tests (including liver function tests, coagulation, and hematology parameters), and vital signs. The investigator also reviewed the local tolerability of the injection by documenting injection-site reactions using a structured assessment. At 0 (predose), 1, 2, 4, 8, and 24 h, the investigator evaluated the injection site for induration, redness, wheal, and pruritus using a four-category scale (none, mild, moderate, severe).

2.5 | PK assessments

Blood samples (4 mL) were collected in lithium heparin at prespecified times for quantitation of rusfertide plasma concentrations; samples were centrifuged to obtain plasma and stored at −80°C. Venous blood and urine samples were used to measure rusfertide concentrations.

PK parameters were estimated using noncompartmental methods (Phoenix WinNonlin v6.3, Certara, Princeton, NJ, USA). Maximum plasma concentration (C_{max}) and time to C_{max} (T_{max}) were observed values. The elimination rate was estimated from the slope of the least squares regression on the terminal log-linear phase. To estimate the area under plasma concentration–time curve (AUC) from time 0 to the last quantifiable concentration (AUC_{0-t}), a linear-up, log-down trapezoidal method was implemented; the result was extrapolated to infinity (AUC_{inf}) by dividing the last quantifiable concentration by the elimination rate; apparent volume of distribution and apparent clearance were also estimated. In addition, the accumulation ratio following repeated administration of rusfertide was estimated by dividing the C_{max} following the second dose by the C_{max} following the first dose. A similar approach was used for AUC_{0-t}. For plasma concentration summary statistics, values below the limit of quantitation were set to 0.

2.6 | PD assessments

Serum iron, serum ferritin, serum transferrin, TSAT, and total iron binding capacity were measured before dosing and at frequent intervals for up to 1 week after single and repeated dosing. Serum iron and TSAT measurements were compared with the rusfertide concentration to assess for the presence of hysteresis. The PD effect of rusfertide, as measured by the area under the effect–time curve (AUEC) for serum iron and TSAT and the AUC, was modeled using an inhibitory sigmoid E_{max} relationship.

2.7 | Immunogenicity assessments

Serum samples were collected prior to dosing on Days 1, 7, and 14 for determination of rusfertide antidrug antibodies. Serum antidrug antibodies were examined via a validated enzyme-linked immunosorbent assay using a three-tiered approach (screening, confirmation, and titration analysis). The sensitivity of the assay, defined as the concentration at which anti-rusfertide antibodies were still detectable, was

354 ng/mL. The samples were analyzed in duplicate; the samples that were less than the plate-specific cut point in the screening phase were reported as negative.

2.8 | Bioanalytical methods

The concentrations of rusfertide in plasma and Triton X-100-stabilized urine were measured using a validated high-performance liquid chromatography–tandem mass spectrometry method. Rusfertide and its stable-isotope-labeled internal standard were isolated from plasma using a protein precipitation procedure; they were isolated from urine using a dilution procedure. Analysis of rusfertide was conducted by high-performance liquid chromatography using an Agilent Zorbax 300 SB C18 column. Samples were analyzed via liquid chromatography coupled with tandem mass spectrometry using an API4000 detector (AB Sciex) using turbospray ion source with positive multiple reaction monitoring.

Calibration curves for rusfertide in human plasma and in urine were linear from 5 to 2000 ng/mL.

2.9 | Statistical methods

This was a first-in-human study, and no formal sample size calculation was conducted. All safety and PK results were reported using descriptive statistics. The safety population comprised all subjects who received at least one dose of rusfertide or placebo; the PK population was all subjects who received rusfertide and had at least one PK parameter.

A power model was used to investigate the dose proportionality of C_{max} and AUC values for rusfertide. This model is defined as $Y = \alpha \cdot (\text{Dose})^{\beta}$, where Y is the PK parameter and β is the proportionality exponent. A value of unity for β indicates dose proportionality. The power model may be linearized by log transformation: $\ln(Y) = \ln(\alpha) + \beta \cdot \ln(\text{Dose})$. PK and PD parameters were summarized using descriptive statistics by dose subgroup, including one pooled placebo group for the single-dose portion of the study. PK and PD data for the repeated dose of rusfertide were summarized similarly.

3 | RESULTS

3.1 | Baseline demographics and clinical characteristics

A total of 56 healthy male subjects were enrolled in the single-dose groups ($n = 45$, rusfertide; $n = 11$, placebo. $n = 8$ per dose in rusfertide 1, 3, 10, 20, 40 mg and $n = 2$ placebo; $n = 5$ in 80 mg rusfertide and $n = 1$ for placebo) and an additional six subjects were enrolled in the repeated-dose groups ($n = 5$, two doses of 40 mg rusfertide; $n = 1$, placebo). Subject baseline demographics and clinical characteristics are summarized in Tables S1 and S2, respectively. Baseline demographics were generally similar across dose groups and clinical characteristics were consistent with healthy status.

3.2 | Tolerability and safety

Three subjects in the single-dose phase completed the 7-day clinical phase of the study involving PK and PD, but they withdrew prior to completing the end-of-study procedures. None of the withdrawals were for safety reasons.

Rusfertide was generally well tolerated. A summary of the TEAEs by dose group is presented in Table 1. There were no serious or severe TEAEs; all TEAEs were mild or moderate in severity and resolved without intervention. A total of 52 TEAEs were reported in 31 subjects receiving rusfertide and 5 TEAEs were noted in four subjects receiving placebo. Of the 52 TEAEs following rusfertide, 23 were injection-site reactions and 30 were considered treatment-related. The incidence of overall TEAEs and injection-site reactions did not increase with increasing dose level. More subjects in the 3 mg rusfertide dose group experienced upper respiratory tract infections; however, the incidence did not increase with rusfertide dose, and no events were noted in the repeated-dose portion of the study.

A total of 12 subjects experienced moderate TEAEs, all of which occurred after rusfertide treatment. All other TEAEs were mild. The frequency of moderate TEAEs increased with increasing dose up to 40 mg rusfertide, but just one was reported at 80 mg. TEAEs seen in two or more subjects following single doses of rusfertide were injection-site erythema (37.8%), headache (17.8%), upper respiratory tract infection (17.8%), injection site pain (4.4%), influenza-like illness (4.4%), and nasal congestion (4.4%) (Table 1).

In the repeated-dose groups, there were 15 TEAEs, none of which occurred in the subject receiving placebo. All five subjects receiving rusfertide reported at least one TEAE. Of the 15 TEAEs, 12 were injection-site reactions experienced by four subjects. The most common TEAEs noted following repeated dosing were injection-site erythema and injection-site induration. In total, two subjects reported three moderate TEAEs, all of which were injection-site erythema. All other TEAEs were mild.

Changes in hemoglobin and reticulocyte counts were small and not consistently related to rusfertide in the single-dose groups (Table S3). All subjects receiving rusfertide doses of >1 mg had platelet count decreases recorded on Day 4 or 5; however, decreases in platelet count were also noted in the placebo group on Day 5. No consistent dose-related effects were observed for all other hematology parameters. No consistent effects were noted in coagulation or clinical chemistry (excluding iron PD) parameters.

There were small declines in hemoglobin on Day 15, and increases in reticulocyte count were noted on Day 21, following two doses of 40 mg rusfertide given 1 week apart. No other consistent effects were observed in hematology, coagulation, or clinical chemistry parameters (Table S3).

There were no consistent or clinically significant changes in vital signs (heart rate, blood pressure) following single and repeated dosing, nor were there any clinically significant abnormal ECG findings in any dose group.

TABLE 1 Summary of treatment-emergent adverse events reported in two or more subjects following subcutaneous rusfertide as an aqueous formulation or placebo.

	Single dose								Repeated dose	
	Rusfertide									
	1 mg ($n = 8$)	3 mg ($n = 8$)	10 mg ($n = 8$)	20 mg ($n = 8$)	40 mg ($n = 8$)	80 mg ($n = 5$)	All doses ($N = 45$)	Placebo ($n = 11$)	2 × 40 mg rusfertide ($n = 5$)	Placebo ($n = 1$)
All TEAEs										
Subjects, *n* (%)	4 (50.0)	5 (62.5)	7 (87.5)	6 (75.0)	5 (62.5)	4 (80.0)	31 (68.9)	4 (36.4)	5 (100)	0
Total events	4	10	11	12	6	9	52	5	15	0
Total unique events	4	7	7	5	3	7	18	4	6	0
Injection-site reaction TEAEs										
Subjects, *n* (%)	1 (12.5)	1 (12.5)	5 (62.5)	6 (75.0)	3 (37.5)	3 (60.0)	19 (42.2)	1 (9.1)	4 (80.0)	0
Total events	1	1	6	7	3	5	23	1	12	0
Total unique events	1	1	2	1	1	4	5	1	3	0
Treatment-related TEAEs										
Subjects, *n* (%)	1 (12.5)	2 (25.0)	6 (75.0)	6 (75.0)	4 (50.0)	4 (80.0)	23 (51.1)	1 (9.1)	4 (80.0)	0
Total events	1	2	7	9	4	7	30	1	13	0
Total unique events	1	2	3	3	2	6	10	1	4	0
General disorders and administrative-site conditions										
Injection-site erythema	0	1 (12.5)	5 (62.5)	6 (75.0)	3 (37.5)	2 (40.0)	17 (37.8)	0	4 (80.0)	0
Injection-site induration	0	0	0	0	0	0	0	0	4 (80.0)	0
Injection-site pain	1 (12.5)	0	0	0	0	1 (20.0)	2 (4.4)	1 (9.1)	1 (20.0)	0
Influenza like illness	0	1 (12.5)	1 (12.5)	0	0	0	2 (4.4)	0	0	0
Nervous system disorders										
Headache	0	2 (25.0)	1 (12.5)	2 (25.0)	1 (12.5)	2 (40.0)	8 (17.8)	1 (9.1)	1 (20.0)	0
Infections and infestations										
Upper respiratory tract infection	1 (12.5)	3 (37.5)	0	1 (12.5)	2 (25.0)	1 (20.0)	8 (17.8)	1 (9.1)	0	0
Respiratory, thoracic, and mediastinal disorders										
Nasal congestion	0	1 (12.5)	0	0	0	1 (20.0)	2 (4.4)	0	0	0

Note: Data are presented as *n* (%) for subjects and *n* for events.
Abbreviation: TEAE, treatment-emergent adverse event.

3.3 | Pharmacokinetics

The mean plasma concentration–time profiles following single doses of rusfertide are presented in Figure 1A. Single-dose PK are summarized in Table 2. Following single subcutaneous doses of rusfertide, median T_{max} occurred at 8 h for 1 mg, 24 h for doses of 3–20 mg, and 2–4.5 h for doses higher than 20 mg. Mean C_{max} increased from 47.4 to 415 ng/mL after single subcutaneous rusfertide doses of 1–80 mg. The terminal half-life increased in a dose-dependent manner, from 17.9 to 52.5 h. Mean apparent clearance was low and rose from 0.5 to 1.4 L/h as the rusfertide dose increased. The mean apparent volume of distribution increased from 12.0 to 102 L over the dose range.

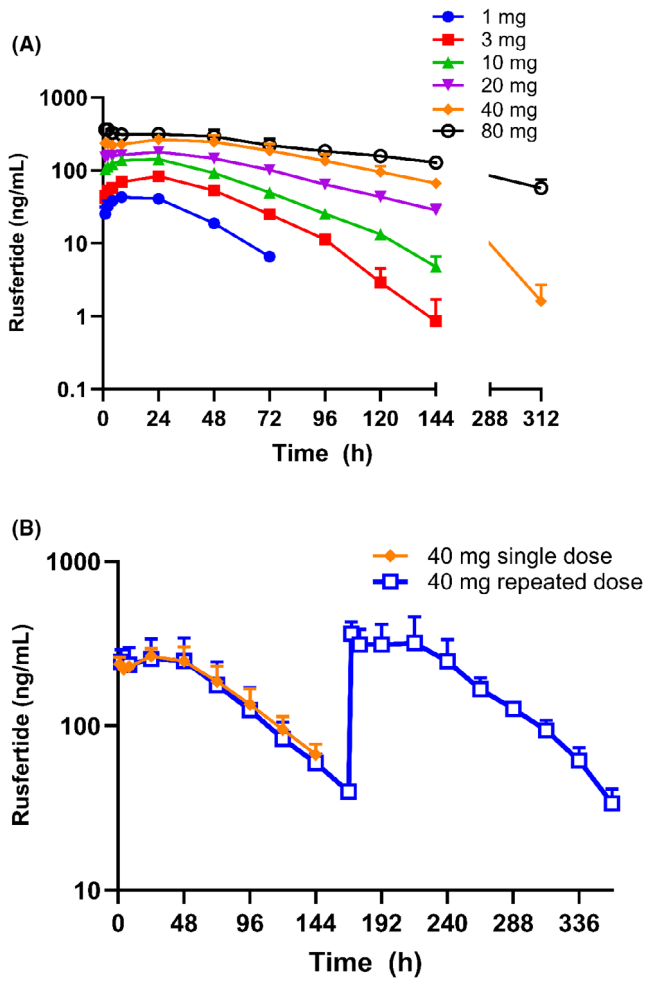

FIGURE 1 Mean rusfertide plasma concentration–time profiles, by dose levels, in healthy subjects following single-dose (A) and repeated-dose (B) subcutaneous administration as an aqueous formulation. Error bars represent standard error of the mean.

Rusfertide concentrations in urine were below the limit of quantitation at all collection periods for all doses.

Figure S1A,B presents the relationship between rusfertide dose and C_{max} and AUC_{inf} following single doses. Dose proportionality was assessed using a power model. The proportionality coefficient was 0.5 (95% confidence interval: 0.43–0.56) for C_{max} and 0.68 (95% confidence interval: 0.60–0.75) for AUC_{inf} (Table S4). The 95% confidence intervals did not include unity, indicating that the increases in C_{max} and AUC_{inf} were not dose proportional over the dose range of 1–80 mg.

The mean plasma concentration–time profile following repeated dosing of rusfertide is presented in Figure 1B. PK parameters after repeated subcutaneous dosing of 40 mg were similar to those after a single 40 mg dose (Table 2). C_{max} and AUC_{0-t} following the second dose were slightly higher than after the first dose. The elimination half-life following repeated dosing was generally similar to that following a single dose, and the accumulation index was 1.5 for both C_{max} and AUC_{0-t} with once-weekly dosing.

3.4 | Pharmacodynamics

The mean PD profiles for serum iron, TSAT, and serum ferritin following single and repeated dosing are presented in Figure 2. Table 3 summarizes the single-dose pharmacodynamics for serum iron, TSAT, serum ferritin, and total iron binding capacity (TIBC). AUEC values of serum iron, TSAT, serum ferritin, and TIBC following repeated dosing of rusfertide are presented in Table S5. Dose-related decreases in serum iron were noted following a single dose of rusfertide (Figure 2A). While the maximum effect (nadir) on serum iron did not change appreciably as the dose increased above 20 mg, higher doses of rusfertide were associated with a longer duration of effect and a slower recovery back to baseline. The time course of mean TSAT is presented in Figure 2B. Similar to the effects seen for serum iron, the nadir on TSAT occurred at 8–48 h, with maximum effect noted at ~20 mg, and higher doses showing a longer duration of effect. A trend for increasing serum ferritin levels over time was observed at rusfertide doses of ≥20 mg, and the effect appeared to be dose-dependent (Figure 2C). The effect on ferritin seemed cumulative with repeated dosing (Table S5), but the levels did start to decrease by the end of the observation period. The effects in the single- and repeated-dose groups were similar, and single-dose PD were predictive of repeated dose effects. The AUEC showed a dose-related effect, with the effect on serum iron and TSAT tapering off at 20 mg (Table 3).

Examination of the relationship between rusfertide plasma concentration–time area under the curve and pharmacodynamics (AUEC) for serum iron (Figure S2A) indicated that the Hill coefficient was ~1 and the average maximum effect was 64%. The AUC that results in 50% of the maximum effect was 13 400 ng h/mL, which is achieved by a dose of 10–20 mg (Table 2). The relationship between AUCt and AUEC for TSAT is presented in Figure S2B. The relationship for TSAT was shallower and more variable than for serum iron; the estimated Hill coefficient for TSAT was 0.73 with a 95% confidence interval including unity. The AUC that results in 50% of the maximum effect on TSAT was 41 200 ng.h/mL, corresponding to a dose range of 40–80 mg (Table 2).

3.5 | Immunogenicity

A total of 238 samples were screened for the presence of anti-rusfertide antibodies. All samples yielded optical density values below their corresponding plate-specific cut points and were considered negative for anti-rusfertide antibodies.

3.6 | Hematology parameters

There was no change in the erythrocyte mean corpuscular hemoglobin, erythrocyte mean corpuscular hemoglobin concentration, erythrocyte mean corpuscular volume, or erythrocyte distribution width following single or repeated dosing of rusfertide in healthy subjects (Table S3).

TABLE 2 Pharmacokinetics of rusfertide aqueous formulation following subcutaneous administration in healthy subjects.

	Single dose						Repeated dose ($n = 5$)	
	1 mg ($n = 8$)	3 mg ($n = 8$)	10 mg ($n = 8$)	20 mg ($n = 8$)	40 mg ($n = 8$)	80 mg ($n = 5$)	Dose 1 (40 mg)	Dose 2 (40 mg)
C_{max} (ng/mL)	47.4 ± 14.3	84.3 ± 31.9	148 ± 51.8	189 ± 33.7	317 ± 127	415 ± 88.1	287 ± 193	418 ± 261
T_{max} (h)	8.0 (4.0, 24.1)	24.0 (8.0, 24.2)	24.0 (8.0, 24.0)	24.0 (2.0, 48.0)	4.5 (1.0, 48.0)	2.0 (1.0, 24.0)	2.0 (2.0, 48.0)	2.0 (2.0, 48.0)
AUC_{0-t} (ng.h/mL	1890 ± 341	4670 ± 990	9070 ± 2230	14 900 ± 2670	26 600 ± 11 500	47 100 ± 11 500	25 600 ± 18 000	35 600 ± 18 200
AUC_{inf} (ng.h/mL)	2240 ± 401	4910 ± 961	9440 ± 2150	16 300 ± 3420	31 300 ± 14 100	59 000 ± 12 900	30 800 ± 18 900	-
$t_{1/2}$ (h)	17.9 ± 2.1	21.3 ± 6.2	26.1 ± 7.0	35.4 ± 9.6	45.0 ± 13	52.5 ± 17	49.6 ± 18	36.1 ± 21
CL/F (L/h)	0.5 ± 0.1	0.6 ± 0.1	1.1 ± 0.2	1.3 ± 0.3	1.4 ± 0.4	1.4 ± 0.3	1.6 ± 0.6	-
Vz/F (L)	12.0 ± 4.0	19.9 ± 8.1	43.2 ± 18	64.0 ± 19	98.5 ± 51	102 ± 10	124 ± 69	-

Note: Data are mean ± SD. Note for T_{max}, median (min, max) is presented.
Abbreviations: AUC, area under the concentration–time curve; AUC_{0-t}, AUC from time 0 to the last quantifiable concentration; AUC_{inf}, AUC from time zero to infinity; CL/F, apparent clearance; C_{max}, maximum observed drug concentration; SD, standard deviation; $t_{1/2}$, apparent elimination half-life; T_{max}, time to the maximum drug concentration; Vz/F, apparent volume of distribution.

4 | DISCUSSION

This first-in-human study demonstrated that rusfertide, a synthetic hepcidin mimetic, at single doses of 1–80 mg was well tolerated in healthy subjects. There were no severe or serious AEs, and no discontinuations due to AEs. Following subcutaneous administration, rusfertide concentrations were detected within 1 h of administration, and median T_{max} was 8–24 h for doses of 1–20 mg and 2–4.5 h for doses of 40 and 80 mg.

Hepcidin is a 25-amino acid peptide hormone that is synthesized primarily by the liver. The hormone contains four disulfide bonds, is prone to aggregation, and is difficult to synthesize; these characteristics render it problematic to develop as a therapeutic drug. Rusfertide is a synthetic peptide hepcidin mimetic that matches the key pharmacophores of the native hormone. It also has desirable drug-like properties including an aqueous solubility >100 mg/mL and human plasma stability >24 h.[35] In preclinical studies, rusfertide reduced cell surface expression of ferroportin with a half-maximal effective concentration of 5 nM and caused >80% reduction in serum iron with a significant sustained reduction for 48 h following a single subcutaneous dose of 1 mg/kg in healthy cynomolgus monkeys.[35] A single subcutaneous dose of 0.3 mg/kg rusfertide in cynomolgus monkeys resulted in 54.9% reduction in serum iron at 24 h after the dose, with serum iron returning to baseline within 60 h. In contrast, a subcutaneous dose of 1 mg/kg in cynomolgus monkeys resulted in ~75.8% reduction in serum iron at 24 h after dosing and had a more sustained effect, with serum iron returning to baseline at ~108 h. With both doses, there was a delay between the maximum rusfertide concentration and maximum PD effect.[41]

This phase 1 study characterized the tolerability, PK, and PD of single and repeated doses of rusfertide following subcutaneous dosing. Rusfertide was well tolerated following single doses of 1–80 mg and repeated doses of 40 mg. Treatment-emergent adverse effects were mild or moderate and were transient. This study suggested no apparent relationship between rusfertide dose and frequency of TEAEs. No clinically relevant changes were noted in clinical chemistry, vital signs, or electrocardiogram assessments following rusfertide administration.

The single-dose PK of subcutaneous rusfertide using an aqueous formulation were characterized by a less than proportional increase in C_{max} and systemic exposure (AUC) over a dose range of 1–80 mg. The apparent volume of distribution was 12–102 L; this was much larger than intravascular volume, and it increased with higher doses. Apparent clearance was low and increased with increasing rusfertide dose. No intact rusfertide was detected in the urine, suggesting the absence of renal clearance.

Following subcutaneous administration of rusfertide, serum iron and TSAT decreased in a dose-dependent manner; these decreases are expected pharmacologic outcomes of iron sequestration into intracellular pools such as splenic macrophages as a result of decreased ferroportin activity. Serum ferritin increased consistently and dose-dependently after single and repeated dosing of rusfertide. The origin and function of serum ferritin remain largely unexplored.[31] Ferritin concentrations vary according to age and sex, and most ferritin is intracellular. The source and secretory pathway for serum ferritin from cells is not completely understood.[42] Because ferritin is an acute-phase reactant protein and serum ferritin arises from tissue ferritin, we suspect that the increased serum ferritin concentrations following rusfertide are a result of iron sequestration in macrophages and ferritin spillover, probably from hepatocytes. The observations of an increase in serum ferritin are consistent with observations in a murine model of PV with a hepcidin mimetic peptide that led to a dose-

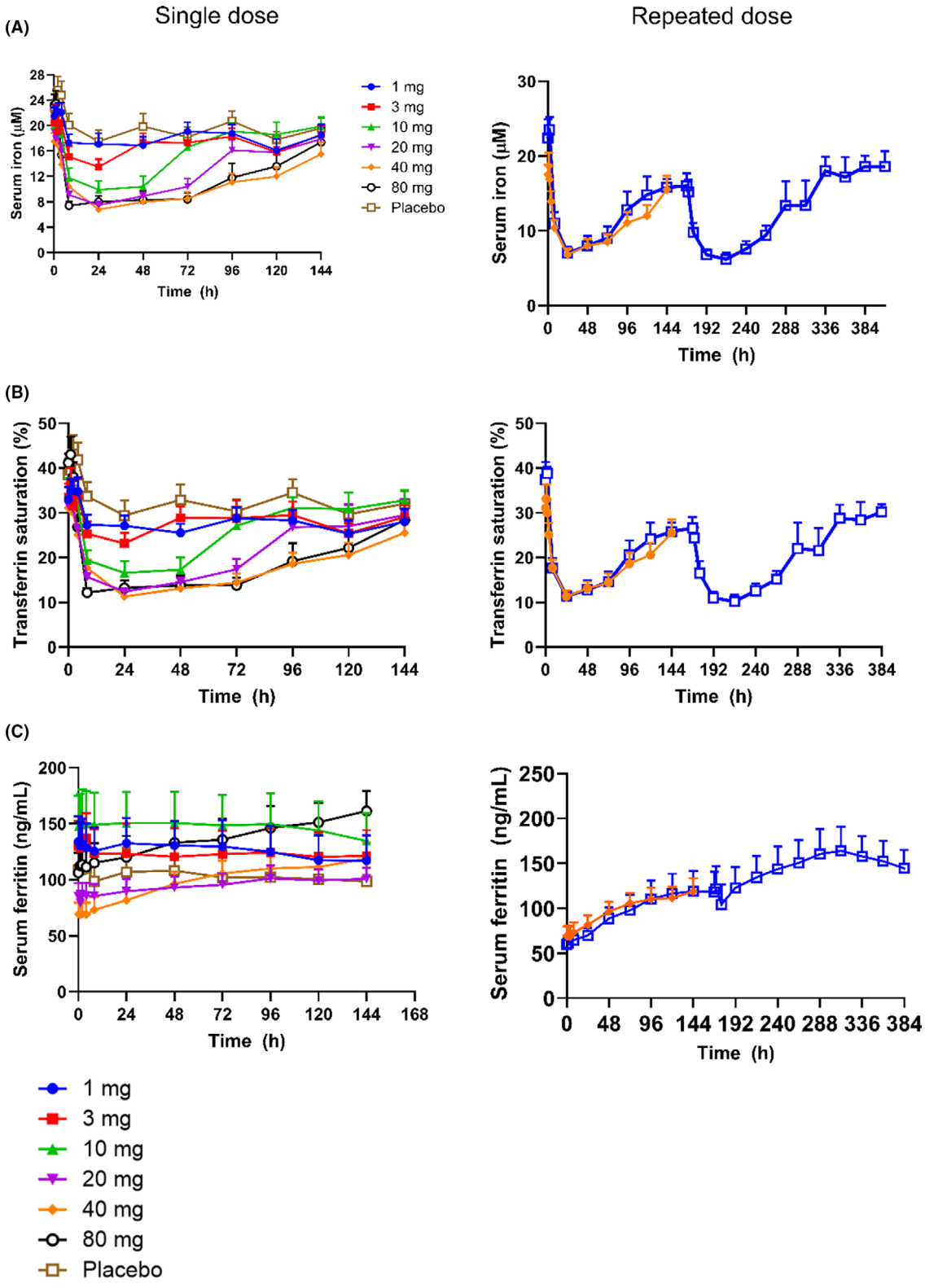

FIGURE 2 Mean pharmacodynamic effect over time following single and repeated subcutaneous dosing of rusfertide aqueous formulation in healthy subjects, by rusfertide dose level: (A) serum iron, (B) transferrin-iron saturation, and (C) ferritin. Error bars represent standard error of the mean.

dependent increase in total iron in the spleen (splenic macrophages) and a corresponding increase in serum ferritin.[30] An increase in serum ferritin was also similarly noted following the administration of LJPC-401, a synthetically produced hepcidin.[43]

TABLE 3 Summary of single-dose pharmacodynamics of rusfertide aqueous formulation following subcutaneous administration.

	Single-dose rusfertide						
	1 mg ($n = 8$)	3 mg ($n = 8$)	10 mg ($n = 8$)	20 mg ($n = 8$)	40 mg ($n = 8$)	80 mg ($n = 5$)	Placebo ($n = 11$)
$AUEC_{0-144}$							
Serum iron (μM h)	2570 ± 491	2410 ± 350	2220 ± 384	1800 ± 457	1470 ± 356	1570 ± 449	2770 ± 638
TSAT (% h)	3950 ± 797	3930 ± 850	3670 ± 743	3000 ± 869	2480 ± 659	2590 ± 801	4610 ± 1150
Ferritin (mg h/L)	18.2 ± 9.1	17.8 ± 9.0	21.2 ± 11	13.7 ± 4.1	14.4 ± 4.6	19.7 ± 6.0	15.0 ± 11
TIBC (μM h/L)	9450 ± 570	8930 ± 733	8800 ± 1130	8810 ± 1390	8570 ± 649	8900 ± 1420	8740 ± 681
ΔE_{max}							
Serum iron (μM)	−8.13 ± 4.3	−6.67 ± 2.3	−11.6 ± 4.3	−11.6 ± 2.6	−11.7 ± 4.0	−16.0 ± 2.4	−8.50 ± 4.5
TSAT (%)	−13.0 ± 7.2	−9.83 ± 3.1	−20.1 ± 6.8	−19.4 ± 4.2	−21.3 ± 6.3	−29.2 ± 2.8	−14.9 ± 8.2
Ferritin (μg/L)	6.75 ± 8.2	9.25 ± 12	12.3 ± 13	21.5 ± 18	50.6 ± 30	55.2 ± 8.4	11.2 ± 17
Time to E_{max} (h)							
Serum iron	36 (0, 120)	24 (8, 72)	16 (8, 120)	24 (8, 24)	24 (4, 72)	8 (8, 24)	60 (8, 120)
TSAT	84 (8, 120)	24 (8, 120)	28 (8, 120)	24 (8, 24)	24 (4, 72)	8 (8, 24)	60 (8, 120)

Note: Data are mean ± SD. Note for Time to E_{max} median (min, max) is presented.
Abbreviations: $AUEC_{0-144}$, area under the effect curve from 0 to 144 h; E_{max}, maximum effect; SD, standard deviation; TIBC, total iron binding capacity; TSAT, transferrin-iron saturation.

Porter et al studied the PK and PD of subcutaneous LJPC-401, a synthetically produced peptide identical to endogenous hepcidin, in healthy subjects.[43] The authors noted dose-related increases in baseline-corrected LJPC-401 concentrations following 4, 10, and 20 mg doses. Peak concentrations occurred at ∼2 h, with concentrations returning to near baseline by ∼24 h for the 4 and 10 mg doses and by 7 days for all dose groups. They also noted that peak effects on serum iron occurred 12 h after dose, with serum iron levels returning to baseline within ∼24 h in the 4 mg group and within ∼48 h in the 10 and 20 mg groups.

Similar to the observations by Porter et al, that hepcidin had rapid clearance and a brief duration of effect, van Eijk et al noted a rapid increase in hepcidin levels, with peak levels occurring 6 h after a dose of lipopolysaccharide in healthy subjects, and levels decreasing to baseline by 24 h.[44] These authors found an initial induction of serum iron, and TSAT peaking ∼3 h after endotoxin administration and subsequently decreasing to below baseline value, reaching a nadir at ∼12 h before starting to return to baseline values.

Hepcidin is reported to have a half-life of a few minutes in cynomolgus monkeys.[14] LJPC-401 (5–30 mg) is reported to have an average half-life of 3.4–11.0 h in healthy subjects.[45] In contrast to hepcidin and LJPC-401, the PK and PD effects of rusfertide appear to be sustained for longer. The sustained effects noted with rusfertide would allow once- or twice-weekly dosing. Clinical studies are currently underway to characterize the safety and efficacy of subcutaneous rusfertide in patients with PV.[46]

No anti-rusfertide antibodies were detected. Rusfertide was generally well tolerated at doses up to 80 mg, with all TEAEs being mild or moderate in nature. Injection-site erythema, injection-site induration, headache, and upper respiratory tract infection were the most common TEAEs. There was no clear relationship between the incidence of TEAEs and rusfertide dose. Injection-site reactions were largely transient without systemic effects and were not considered clinically concerning or limiting to dose escalation.

5 | CONCLUSIONS

Subcutaneous rusfertide as an aqueous formulation was generally well tolerated in this first-in-human study. TEAEs were mild or moderate and were transient. No patterns indicative of clinically important AEs, laboratory abnormalities, or changes in vital signs or ECG were observed and no serious or severe AEs were reported. There was a dose-related, but less than proportional, increase in exposure as measured by C_{max} and AUC over the dose range of 1–80 mg. Dose-related PD effects were noted in serum iron and TSAT. PK and PD results from the current study provide a rationale for dose selection in patients.

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Protagonist Therapeutics Inc. sponsored this study. The sponsor was involved in the study design and collection, analysis, and interpretation of data, as well as data checking of information provided in the manuscript. Ultimate responsibility for opinions, conclusions, and data interpretation remained with the authors. The corresponding author had full access to all data in the study and

had final responsibility for the decision to submit this paper for publication.

CONFLICT OF INTEREST STATEMENT

Nishit B. Modi and Suneel Gupta are employees of Protagonist Therapeutics Inc., the study sponsor. Jason D. Lickliter is an employee of Nucleus Network. No other potential conflicts of interest were declared.

DATA AVAILABILITY STATEMENT

Data requests from external parties will be considered on a case-by-case basis. Protagonist Therapeutics Inc. reserves the right to deny requests for any and all appropriate reasons. Data requests that risk sharing participant-level data or proprietary information will not be approved.

CONSENT STATEMENT

All subjects gave written informed consent to participate in the study.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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