Rusfertide for the treatment of iron overload in HFE-related haemochromatosis: an open-label, multicentre, proof-of-concept phase 2 trial

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Summary

Background Hereditary haemochromatosis protein (HFE)-related haemochromatosis, an inherited iron overload disorder caused by insufficient hepcidin production, results in excessive iron absorption and tissue and organ injury, and is treated with first-line therapeutic phlebotomy. We aimed to investigate the efficacy and safety of rusfertide, a peptidic mimetic of hepcidin, in patients with HFE-related haemochromatosis.

Methods This open-label, multicentre, proof-of-concept phase 2 trial was done across nine academic and community centres in the USA and Canada. Adults (aged ≥18 years) with HFE-related haemochromatosis on a stable therapeutic phlebotomy regimen (maintenance phase) for at least 6 months before screening and who had a phlebotomy frequency of at least 0·25 per month (eg, at least three phlebotomies in 12 months or at least four phlebotomies in 15 months) and less than one phlebotomy per month, with serum ferritin of less than 300 ng/mL and haemoglobin of more than 11·5 g/dL, were eligible. Patients initiated 24 weeks of subcutaneous rusfertide treatment within 7 days of a scheduled phlebotomy at 10 mg once weekly. Rusfertide doses and dosing schedules could be adjusted to maintain serum transferrin iron saturation (TSAT) at less than 40%. During rusfertide treatment, investigators were to consider the need for phlebotomy when the serum ferritin and TSAT values exceeded the patient's individual pre-phlebotomy serum ferritin and TSAT values. No primary endpoint or testing hierarchy was prespecified. Prespecified efficacy endpoints included the change in the frequency of phlebotomies; the proportion of patients achieving phlebotomy independence; change in serum iron, TSAT, serum transferrin, serum ferritin, and liver iron concentration (LIC) as measured by MRI; and treatment-emergent adverse events (TEAEs). The key efficacy analyses for phlebotomy rate and LIC were conducted by use of paired *t* tests in the intention-to-treat population, defined as all patients who received any study drug and who had pretreatment and at least one post-dose measurement. We included all participants who received at least one dose of rusfertide in the safety analyses. This trial is closed and completed and is registered with ClinicalTrials.gov, NCT04202965.

Findings Between March 11, 2020, and April 23, 2021, 28 patients were screened and 16 (ten [63%] men and six [38%] women) were enrolled. 16 were included in analyses of phlebotomy endpoints and 14 for the LIC endpoint. 12 (75%) patients completed 24 weeks of treatment. The mean number of phlebotomies was significantly reduced during the 24-week rusfertide treatment (0·06 phlebotomies [95% CI −0·07 to 0·20]) compared with 24 weeks pre-study (2·31 phlebotomies [95% CI 1·77 to 2·85]; p<0·0001). 15 (94%) of 16 patients were phlebotomy-free during the treatment period. Mean LIC in the 14 patients in the intention-to-treat population was 1·4 mg iron per g dry liver weight (95% CI 1·0 to 1·8) at screening and 1·1 mg iron per g dry liver weight (95% CI 0·9 to 1·3) at the end of treatment (p=0·068). Mean TSAT was 45·3% (95% CI 33·2 to 57·3) at screening, 36·7% (24·2 to 49·2) after the pretreatment phlebotomy, 21·8% (15·8 to 27·9) 24 h after the first dose of rusfertide, 40·4% (27·1 to 53·8) at the end of treatment, and 32·6% (25·0 to 40·1) over the treatment duration. Mean serum iron was 24·6 µmol/L (95% CI 18·6 to 30·6), 20·1 µmol/L (14·8 to 25·3), 11·9 µmol/L (9·2 to 14·7), 22·5 µmol/L (15·9 to 29·1), and 19·0 µmol/L (15·3 to 22·6) at these same timepoints, respectively. Mean serum ferritin was 83·3 µg/L (52·2 to 114.4), 65·5 µg/L (32·1 to 98·9), 62·8 µg/L (33·8 to 91·9), 150·0 µg/L (86·6 to 213.3), and 94·3 µg/L (54·9 to 133.6) at these same timepoints, respectively. There were only minor changes in serum transferrin concentration. 12 (75%) patients had at least one TEAE, the most common of which was injection site pain (five [31%] patients). All TEAEs were mild or moderate in severity, except for a serious adverse event of pancreatic adenocarcinoma, which was considered severe and unrelated to treatment and was pre-existing and diagnosed 21 days after starting rusfertide treatment.

Interpretation Rusfertide prevents iron re-accumulation in the absence of phlebotomies and could be a viable therapeutic option for selected patients with haemochromatosis.

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A complete list of study investigators is provided in the appendix (p 2)

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See Online for appendix

Research in context

Evidence before this study

We searched PubMed on May 6, 2023, for articles published since Jan 1, 2018, without language restrictions, using the search terms "hereditary hemochromatosis" AND ("treatment" OR "efficacy"). Patients with haemochromatosis, an inherited iron overload disorder mostly due to deficiency of hepcidin, are at increased risk of mortality and morbidity. The current first-line treatment is therapeutic phlebotomy to mobilise excess body iron. Although phlebotomy is effective and generally well tolerated, some patients find it very inconvenient and compliance decreases with time. Patients with underlying anaemia, heart disease, or poor venous access are also not candidates for therapeutic phlebotomy. There are currently no approved pharmacotherapeutic treatments. Although iron chelators can be used, they are associated with side-effects, including hepatic and renal toxicity, and are only recommended for patients who are intolerant or refractory to therapeutic phlebotomies. Non-clinical data in hepcidin-deficient mouse models of haemochromatosis indicate that hepcidin and hepcidin mimetics can decrease iron absorption and iron loading.

Added value of this study

To our knowledge, this open-label, phase 2 trial is the first to assess the efficacy and safety of subcutaneous rusfertide, a novel peptidic hepcidin mimetic, administered for 24 weeks in patients with haemochromatosis on maintenance therapeutic phlebotomies. Rusfertide treatment rapidly reduced serum iron concentrations and transferrin iron saturation, significantly reduced the rate of phlebotomy compared with pre-study values, and limited liver iron accumulation. Rusfertide treatment was generally well tolerated in this patient population. Treatment-emergent adverse events were mostly mild or moderate and the most common treatment-emergent adverse events were injection site reactions. The current findings indicate that hepcidin mimetics might offer an alternative mode of therapy for managing iron accumulation in patients who are unwilling or unable to undergo therapeutic phlebotomies.

Implications of all the available evidence

Results from this open-label study indicate that rusfertide might offer an alternative approach to managing iron accumulation in patients with haemochromatosis who are unwilling or unable to undergo therapeutic phlebotomies. As our study was done in a small group of patients on maintenance phlebotomies without a placebo control group, larger, placebo-controlled studies are needed to fully define and characterise the role for rusfertide in treating patients with haemochromatosis.

Introduction

Haemochromatosis is an inherited iron overload disorder characterised by excessive absorption of dietary iron.[1] Haemochromatosis affects approximately one in 150 to 200 people of northern European descent.[2–4] The most common form of haemochromatosis in people of northern European ancestry is due to the initial detection of a homozygous mutation in homeostatic iron regulator (*HFE*; hereditary hemochromatosis protein), primarily a single nucleotide change (845G→A), causing substitution of cysteine by tyrosine at amino acid 282 (Cys282Tyr variant).[5] Subsequently, a second minor *HFE* polymorphism (His63Asp variant) was detected and other studies have indicated that the Cys282Tyr variant is rare in some regions of the world.[6–9] The current consensus is that the genetic basis of haemochromatosis is more heterogeneous than previously thought and that variants in other iron-controlling genes are also associated with haemochromatosis.[5] People with haemochromatosis have insufficient hepcidin production or, rarely, resistance to hepcidin, resulting in hyperabsorption of dietary iron, increased release of iron from recycling macrophages, increased plasma iron concentrations and transferrin iron saturation (TSAT), and iron overload.[5,10] Iron overload in organs, such as the pancreas, liver, heart, and endocrine glands, can lead to diabetes, cirrhosis, and cardiac failure.

Hepcidin, a 25-amino acid peptide hormone produced primarily by the liver, is considered the principal regulator of extracellular iron homoeostasis.[11] Hepcidin binds to ferroportin, inducing endocytosis and subsequent lysosomal degradation of ferroportin.[12,13] Hepcidin acts by regulating iron influx into plasma from tissues involved in iron storage or transport—eg, duodenal enterocytes involved in dietary iron absorption, hepatocytes that store iron, and macrophages in the spleen and liver that recycle iron from senescent erythrocytes.[14]

The primary goal in the management of haemochromatosis is to identify patients before end-organ injury and to initiate treatment before irreversible organ damage occurs.[15] Iron depletion via therapeutic phlebotomy is currently the recommended first-line treatment.[15] Although therapeutic phlebotomy is effective and generally well tolerated, 26 (25%) of 102 patients with haemochromatosis undergoing maintenance phlebotomy treatment described it as inconvenient or very inconvenient.[16] Patient compliance also declines with time,[17] and some patients are not candidates for therapeutic phlebotomy due to underlying anaemia, heart disease, or poor venous access.

Although iron chelators, such as deferasirox, have been reported to reduce iron burden in patients with haemochromatosis,[18,19] they are only recommended for patients who cannot tolerate therapeutic phlebotomy or when phlebotomies could cause harm.[15] Treating patients with haemochromatosis with hepcidin could potentially eliminate the need for therapeutic phlebotomy. However, synthesis of full-length hepcidin is relatively inefficient

and synthetic hepcidin has poor pharmaceutical properties and a rapid systemic clearance.[20] Various minihepcidins, short peptides based on the N-terminal structure of hepcidin, have shown biological efficacy and have been evaluated in non-clinical mouse models of haemochromatosis.[20–26] One study tested the ability of a minihepcidin to prevent iron loading by iron-depleting hepcidin-deficient mice before switching them to an iron-loading diet with injections of the minihepcidin or solvent (control).[22] They found that the minihepcidin decreased iron loading in the liver and heart compared with the solvent control. Thus, a hepcidin mimetic might supplement hepcidin concentrations, reducing hyperabsorption of iron and preventing iron overload. Rusfertide (also known as PTG-300) is a peptidic agent structurally related to hepcidin that mimics the inhibitory actions of hepcidin on ferroportin.[24–26] We aimed to investigate the efficacy and safety of rusfertide in patients with HFE-related haemochromatosis.

Methods

Study design and participants

This open-label, multicentre, phase 2, proof-of-concept trial was designed to characterise the safety and efficacy of rusfertide in patients with HFE-related haemochromatosis who were in the maintenance phase of phlebotomy therapy. The study was conducted across nine academic and community centres in the USA and Canada (appendix p 2). We included male and female patients aged 18 years or older with a confirmed diagnosis of HFE-related haemochromatosis; who were in the maintenance phase of treatment, with documented regimens of stable phlebotomies for at least 6 months before screening; who had a phlebotomy frequency of at least 0·25 per month (eg, at least three phlebotomies in 12 months or at least four phlebotomies in 15 months) and less than one phlebotomy per month; and whose serum ferritin at screening was less than 300 ng/mL and haemoglobin at screening was more than 11·5 g/dL. We excluded patients who had clinically meaningful laboratory abnormalities, were receiving iron chelation therapy or erythrocytapheresis, or who had clinically significant organ damage from iron overload, as judged by the investigator. Women of childbearing potential and men with partners of childbearing potential were required to use adequate contraception during the trial and for 28 days after the last dose of study drug for women and 90 days after the last dose for men.

The study protocol, consent form, and patient information sheet were reviewed and approved by local independent ethics committees and review boards. Study patients provided written, informed consent before participating in the trial. The trial was conducted in compliance with the ethical principles of the Declaration of Helsinki and the International Conference on Harmonization Good Clinical Practice guideline. An optional ^{57}Fe absorption substudy was included (appendix p 32); however, no participants were enrolled in this substudy. A full version of the protocol is available in the appendix (pp 7–67).

Procedures

Patients underwent screening for eligibility within 21 days before starting rusfertide treatment. Participants self-identified their gender with options of male and female. Patients were required to provide records of their previous phlebotomies and the rate was calculated from this historical chart record. Patients began rusfertide treatment within 7 days of a scheduled phlebotomy. Physicians used their medical judgement in determining when to phlebotomise their patients before the study. Treatment with rusfertide was initiated at 10 mg per week. Patients received subcutaneous injections of rusfertide for 24 weeks, with a follow-up visit 4 weeks after the final dose. Dose escalation and adjustment were guided by tolerability and changes in TSAT. Rusfertide dosing could be adjusted to achieve a TSAT of less than 40% approximately 1 day after a dose, the expected nadir, and before the next dose. A TSAT of more than 50% in men and more than 45% in women has been defined as elevated in haemochromatosis.[4] We chose a target TSAT of less than 40% for dose selection to account for potential clinical and diurnal variability. Investigators could consider once-weekly or twice-weekly dosing to achieve this goal. During rusfertide treatment, investigators were advised to consider the need for phlebotomy when the serum ferritin and TSAT values exceeded the patient's individual pre-phlebotomy serum ferritin and TSAT values. Phlebotomy was based on the investigators medical judgement. Patients were trained to self-administer the subcutaneous injections at home and were instructed to continue their individual iron-restricted diet during the study. Injections during clinic visits were done in the clinic after measurement of TSAT and serum iron. Compliance with treatment was assessed at every visit by the return of used kits.

Following enrolment, patients were assessed before the first dose, 1 day following the first dose and on weeks 2, 4, 8, 12, 16, 20, and 24 with a safety follow-up at week 28 (appendix pp 38–40). Investigators could implement additional visits to facilitate dose adjustment. Serum iron, ferritin, transferrin, and TSAT were measured at screening (defined as the prestudy baseline), before the first dose (pre-dose) and 24 h after (post-dose) the first dose, and before every dose (pre-dose) at each scheduled clinic visit. Venous blood samples were collected just before the first dose and 24 h after the first dose, pre-dose at all clinic visits, and at the week 28 safety follow-up for measurement of plasma concentrations of rusfertide. Plasma concentrations of rusfertide were quantified by use of a validated liquid chromatography with tandem mass spectrometry method (appendix p 3). Liver iron concentration (LIC) was assessed during the screening period before starting rusfertide treatment and

at the end of rusfertide treatment (week 24 or at early termination) by MRI. Serum samples for assessment of anti-drug antibodies (ADAs) were collected before the first dose, at weeks 8, 16, and 24, and at the safety follow-up at week 28. If a patient developed ADAs, an assessment was made to establish whether these ADAs were neutralising and their impact on safety and efficacy was evaluated. In addition, as a post-hoc assessment, non-transferrin-bound iron (NTBI) was measured at screening, week 8, and week 28. NTBI measurements were done by use of a chelation-ultrafiltration-detection approach (appendix p 4).

Additional assessments included patient-reported outcomes on the Short Form Health Survey 36 (SF-36) and Patient Global Impression of Change (PGI-C). Patients completed the SF-36 at screening and at the end of their study treatment. The SF-36 is a questionnaire of health status based on 36 questions distributed into eight domains of activities of daily life that are frequently evaluated as they are influenced by disease (appendix pp 66–72). These domains consist of physical functioning, role-physical, bodily pain, general health perception, vitality, social functioning, role-emotional, and mental health. Domain scores range from 0 to 100, with higher scores indicating better health, and result in two summary measures of physical (Physical Component Summary [PCS]) and mental (Mental Component Summary [MCS]) health. Patients completed the PGI-C (appendix p 73) after 8 weeks, 16 weeks, and 24 weeks of rusfertide treatment or at early termination. The PGI-C is a 7 point scale on which the participant rates their improvement in overall symptoms relative to their condition before starting the study, ranging from 1 (very much improved) to 7 (very much worse), with 4 reflecting no change.

Safety assessments included adverse events (AEs; screening, all clinic visits, and week 28 follow-up), physical examinations (screening and week 28 follow-up), vital signs (blood pressure, heart rate, respiratory rate, and body temperature; screening, all clinic visits but week 2), clinical laboratory tests (chemistry and haematology, screening, before the first dose and weeks 4, 8, 12, 16, 20, 24, and 28; and urinalysis on weeks 4, 12, and 20), and 12-lead electrocardiograms (ECGs; screening, before the first dose and 24 h following the first dose, and weeks 8, 16, 24, and 28). AEs, either observed by the investigator or reported by patients, were evaluated for severity according to the Common Terminology Criteria for Adverse Events (version 5.0), and for possible relationship to study treatment.

Outcomes

This was a proof-of-concept study and no primary endpoint or testing hierarchy was prespecified. Efficacy was based on multiple prespecified endpoints: change in the frequency of phlebotomies during treatment compared with the pre-study frequency of phlebotomies (24 weeks); proportion of patients who achieved phlebotomy independence; time to first phlebotomy after dosing; change from baseline in LIC; change from baseline in serum ferritin, serum transferrin, serum iron, and TSAT; and the duration of time that serum ferritin and TSAT values were less than their baseline values. Other prespecified endpoints were rusfertide pharmacokinetics (summarised using concentrations 24 h after the first dose and the pre-dose trough concentrations); the MCS and PCS measures of the SF-36; the PGI-C; the incidence of ADAs; treatment-emergent AEs (TEAEs) and serious AEs; treatment-related TEAEs and serious AEs; TEAEs leading to study discontinuation; and vital signs, laboratory assessment, ECG, and physical examination findings. Change in the concentration of soluble transferrin receptor from baseline was a prespecified endpoint but is not reported herein because a technical oversight prevented measurement. Similarly, change in iron absorption following administration of rusfertide is not reported because no participants were enrolled in the iron absorption substudy. In post-hoc analyses, changes in NTBI concentration and the duration of time that serum iron concentration was less than the baseline values were also estimated.

Statistical analysis

This trial was exploratory and no formal criteria for sample size were prespecified. The sample size was based on a risk–benefit assessment. Assuming a baseline phlebotomy frequency of two to six phlebotomies per year,[27] a sample size of 20 patients would provide at least 80% power to detect a reduction of one to three phlebotomies per year. The power calculation was based on a hypothesised effect size of 0·5 (mean change 1 [SD 2] phlebotomy) during 24 weeks using a one-sided one-sample *t* test with an α level set at 10%. Assuming a 25% dropout, 28 participants were planned to be recruited. Demographic and disease characteristics are summarised by use of descriptive statistics (mean, with SD or 95% CI) for continuous variables and by use of frequency and percentage for discrete variables. The mean weekly phlebotomy rate (number of phlebotomies per week) was estimated based on the number of phlebotomies received by the patient over a 24 week interval during the study treatment and based on the prestudy phlebotomy history. Descriptive statistics were calculated and summarised for all endpoints. The key efficacy analyses for phlebotomy rate and LIC were conducted by use of paired *t* tests in the intention-to-treat population, defined as all patients who had prescreening and at least one post-dose measurement. Post-hoc analysis of LIC was also done in the modified intention-to-treat population, defined as all patients who completed 24 weeks of treatment and had prescreening and end-of-study measurements. The effect of rusfertide on the relationship between the change in NTBI and in TSAT

was examined as a post hoc analysis. The assumption of normality for paired *t* tests was assessed by use of the Shapiro-Wilk test and the LIC data were shown to pass normality. Correlation analyses were based on Spearman correlation. Safety was assessed in all patients who received at least one dose of rusfertide. All statistical tests were two-sided, with p<0·05 determining significance. Statistical analyses were done with SAS (version 9.4) and GraphPad (Prism 9). No data safety monitoring committee was used. No analysis by gender was planned in this proof-of-concept study. The trial is closed and completed and is registered with ClinicalTrials.gov, NCT04202965.

Role of the funding source

The funder of the study was responsible for study design, preparing the trial protocol, data collection, data analysis, interpretation, and writing of the report.

Results

From March 11, 2020, to April 23, 2021, 28 patients with haemochromatosis were screened and 16 were enrolled in the study. The study was completed on Oct 6, 2021. 16 patients were included in the intention-to-treat population for phlebotomy endpoints, 14 patients for LIC (two patients did not have an end-of-study MRI), and 11 patients in the modified intention-to-treat population. 12 (75%) of the 16 patients enrolled completed 24 weeks of dosing. Three (19%) patients discontinued the study (following 4 weeks, 7 weeks, and 8 weeks of treatment, respectively) due to adverse events and one (6%) patient was discontinued for not meeting inclusion or exclusion criteria. No patients were discontinued for non-compliance. Although we intended to enrol 28 patients so that at least 20 would complete the study, only 16 patients were enrolled; further enrolment was stopped due to COVID-19 restrictions and because the evolving data allowed adequate assessment of safety and treatment effect in this proof-of-concept study.

15 (94%) of 16 participants were White and ten (63%) were male (table 1). Mean age was 62·5 years (SD 12·3), with six (38%) patients aged 65 years or older. Of the 12 patients who reported their *HFE* genotype, five (42%) were Cys282Tyr homozygous, six (50%) were Cys282Tyr and His63Asp compound heterozygous, and one (8%) was His63Asp homozygous. Median duration of disease since diagnosis was 13 years (IQR 11–16). These baseline characteristics are reflective of a population of patients with haemochromatosis in the maintenance phase of treatment with therapeutic phlebotomy.

The mean duration of treatment in the study was 19·8 weeks (SD 8·4). Patients' weekly dose was titrated to achieve a target TSAT of less than 40%. The mean weekly rusfertide dose at the end of the trial was 19·4 mg (SD 10·0). Of the 12 patients who completed the 24 week treatment, eight (67%) were on a twice-weekly dosing regimen, which either comprised 10 mg twice weekly (three [25%] patients) or twice-weekly alternating doses of 10 mg and 20 mg (four [33%] patients) or 20 mg and 40 mg (one [8%] patient). Four (33%) patients were on a 10 mg (three patients) or 20 mg (one patient) once-weekly regimen.

	Patients (n=16)
Age, years	62·5 (12·3)
Aged ≥65 years	6 (38%)
Weight, kg	88·1 (18·6)
Gender	
Male	10 (63%)
Female	6 (38%)
Race	
White	15 (94%)
Other or not specified	1 (6%)
Number of phlebotomies in 24 weeks before enrolment	2·3 (1·0)
3–4 phlebotomies	7 (44%)
1–2 phlebotomies	9 (56%)
Time between phlebotomies in 24 weeks before enrolment, days	91 (83–143)
Time since diagnosis*, years	13 (11–16)
Serum iron, µmol/L	24·6 (11·2)
Serum transferrin, µmol/L	28·8 (5·1)
Serum ferritin, µg/L	83·3 (58·3)
Number of patients with serum ferritin >50 µg/L	9 (56%)
Transferrin iron saturation, %	45·3% (22·7)
C-reactive protein, mg/L	1·3 (1·1)
Liver iron concentration, mg iron per g dry liver weight	1·4 (0·6)
Non-transferrin-bound iron†, µmol/L	0·93 (0·88)
Short Form Health Survey 36	
Mental Component Summary	51·6 (9·7)
Physical Component Summary	49·3 (7·5)

Data are mean (SD), n (%), or median (IQR). *n=9. †n=14.

Table 1: **Demographics and disease characteristics**

15 (94%) of the 16 patients enrolled had no phlebotomies during the treatment period and only one (6%) patient, a 77-year-old man, underwent therapeutic phlebotomy 56 days after starting rusfertide therapy (figure 1). On day 29, the closest assessment before the phlebotomy, his serum ferritin was 41·4 µg/L, serum iron was 20·6 µmol/L, serum transferrin was 26·5 µmol/L, and TSAT was 39%, all within range for a patient with well controlled haemochromatosis. In the study population (n=16), the mean number of phlebotomies was 0·06 (95% CI –0·07 to 0·20) during the 24-week treatment period with rusfertide, compared with 2·31 (1·77 to 2·85) during the 24 weeks before study enrolment (p<0·0001). The mean weekly phlebotomy rate was 0·096 phlebotomies per week (95% CI 0·074 to 0·119) pre-study and 0·003 phlebotomies per week (–0·003 to 0·008) during treatment with rusfertide (p<0·0001). As only one patient had therapeutic phlebotomy, we could not analyse median time to first phlebotomy.

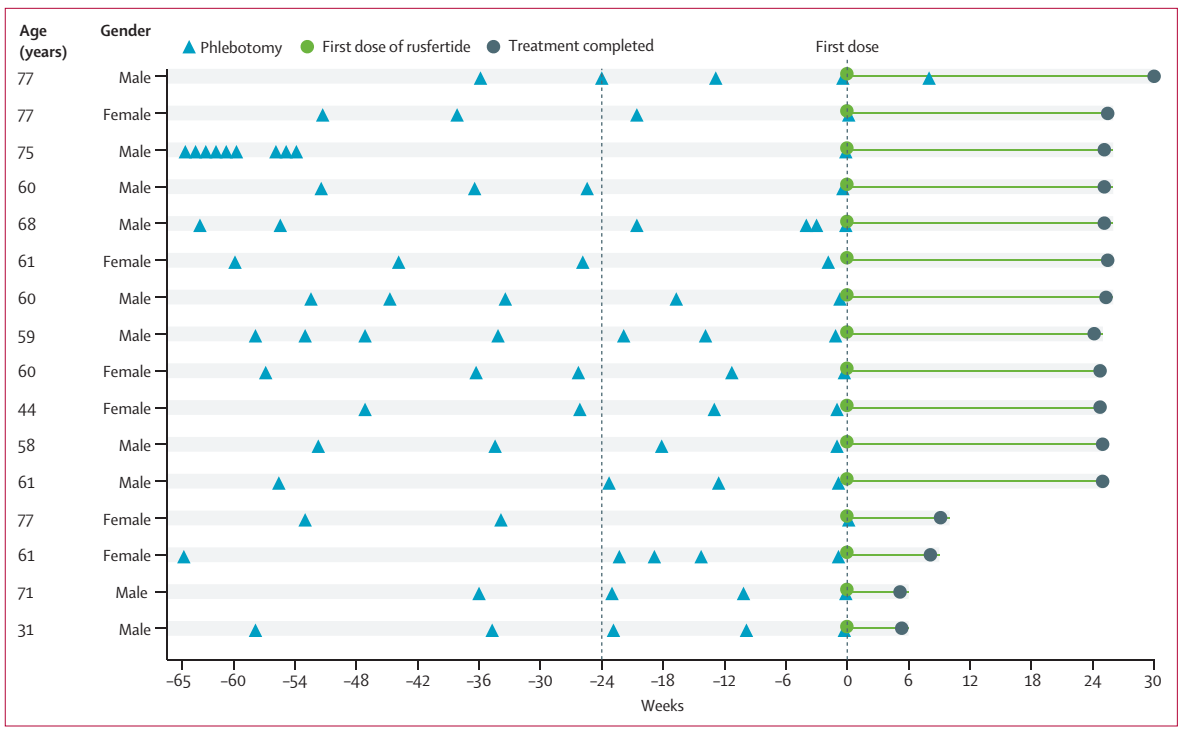

Figure 1: **Effect of rusfertide on phlebotomy rate**

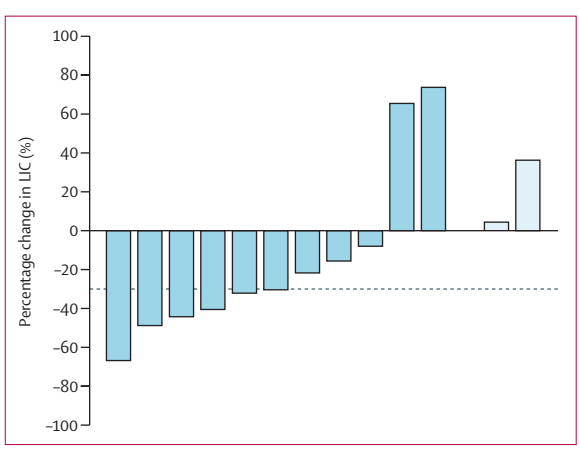

Figure 2: **Waterfall plot of the percentage change in LIC following treatment with rusfertide**

The dashed horizontal line depicts a 30% decline in LIC. Each bar represents an individual patient. Two patients did not have an end-of-study MRI and are not shown. Three patients who received less than 24 weeks of rusfertide treatment are shown as light blue bars (one patient had no change in LIC). The two patients who received at least 24 weeks of rusfertide treatment and had a high LIC at the end of the study had the lowest LICs at screening. LIC=liver iron concentration.

Mean LIC by MRI in the 14 patients who had paired measurements before and after treatment was 1·4 mg iron per g dry liver weight (95% CI 1·0–1·8) at screening and 1·1 mg iron per g dry liver weight (0·9–1·3) at the end of treatment (p=0·068). Six (43%) of the 16 patients had at least a 30% reduction in LIC (figure 2). In a post-hoc analysis considering the 11 patients who completed 24 weeks of treatment with rusfertide and had paired measurements, mean LIC decreased from 1·5 mg iron per g dry liver weight (95% CI 1·1–2·0) at screening to 1·1 mg iron per g dry liver weight (0·8–1·4) at the end of treatment (p=0·042).

Mean serum iron (figure 3A) at screening was 24·6 µmol/L (95% CI 18·6–30·6), which decreased to 20·1 µmol/L (14·8–25·3) after the pretreatment phlebotomy. Following administration of a single 10 mg dose of rusfertide, serum iron decreased to 11·9 µmol/L (9·2–14·7) 24 h after the dose. Mean serum iron at the end of treatment, reflecting the minimum drug effect before the next rusfertide dose, was 22·5 µmol/L (15·9–29·1) and mean serum iron across the duration of treatment with rusfertide was 19·0 µmol/L (15·3–22·6). For the 12 patients who completed 24 weeks of treatment with rusfertide, in a post-hoc analysis, the estimated median duration of time that serum iron concentration was less than screening values was 14 weeks (IQR 10–21).

Similar to observations with serum iron, mean TSAT (figure 3B) was 45·3% (95% CI 33·2–57·3) at screening, which decreased to 36·7% (24·2–49·2) after the pretreatment phlebotomy. Following a single 10 mg dose of rusfertide, mean TSAT decreased to 21·8% (15·8–27·9) 24 h after the dose. TSAT at the end of treatment was 40·4% (27·1–53·8) and mean TSAT over the treatment duration was 32·6% (25·0–40·1). For the 12 patients who completed 24 weeks of treatment with rusfertide, the estimated median duration of time that TSAT was less than screening values was 15 weeks (IQR 10–21). There were

minor changes in serum transferrin concentration (figure 3C). Serum transferrin was 28·8 µmol/L (26·1–31·5) at screening and 29·7 µmol/L (26·3–33·1) following the pretreatment phlebotomy. After a single 10 mg dose of rusfertide, serum transferrin was 29·5 µmol/L (26·4–32·5) 24 h after the dose. Serum transferrin concentration at the end of treatment was 29·7 µmol/L (26·7–32·7) and mean serum transferrin concentration over the treatment duration was 30·8 µmol/L (27·9–33·6). Mean serum ferritin (figure 3D) was 83·3 µg/L (52·2–114·4) at screening and 65·5 µg/L (32·1–98·9) following a phlebotomy. There was only a slight decrease in serum ferritin 24 h following a 10 mg dose of rusfertide (mean 62·8 µg/L [33·8–91·9]); the trough serum ferritin had increased to 150·0 µg/L (86·6–213·3) at the end of the study and the mean serum ferritin concentration over the treatment duration was 94·3 µg/L (54·9–133·6). For the 12 patients who completed 24 weeks of treatment, the median duration of time that serum ferritin concentration was less than screening values was 6 weeks (IQR 2–14).

In post-hoc analyses, mean NTBI concentration was 0·9 µmol/L (95% CI 0·4–1·4; 14 patients) at screening, 0·6 µmol/L (0·4–0·7; 14 patients) following 8 weeks of treatment with rusfertide, and 1·2 µmol/L (95% CI 0·2–2·1; 12 patients) at the safety follow-up visit 4 weeks after the final dose of rusfertide. For the six patients who had TSAT values of more than 50% at screening and NTBI measurements pretreatment, five had NTBI concentrations of equal to or more than the lower limit of quantitation (0·47 µmol/L). Three of these six patients had TSAT values of less than 50% following 8 weeks of rusfertide treatment and all three patients had NTBI concentrations of less than 0·47 µmol/L following 8 weeks of treatment with rusfertide. There was a good correlation between TSAT and NTBI (Spearman's correlation r=0·624, p=0·0004; figure 4).

Following the 10 mg rusfertide starting dose, the mean rusfertide plasma concentration at 24 h was 74·6 ng/mL (SD 34·4; 16 patients). The mean trough concentration was 20·0 ng/mL (SD 49·1; 16 patients) after 4 weeks of treatment and 46·2 ng/mL (55·3; 15 patients) at the end of rusfertide treatment (appendix p 5). No treatment-emergent ADAs against rusfertide were detected.

At the end of treatment with rusfertide, seven (58%) of the 12 patients who completed 24 weeks of treatment indicated an improvement in the PGI-C, three (25%) indicated no change, and two (17%) indicated minimal worsening (figure 5). At screening (16 patients), the mean SF-36 PCS was 49·3 (SD 7·5) and the mean MCS was 51·6 (9·7). At the end of study treatment with rusfertide (15 patients), the mean PCS was 51·8 (6·6) and the mean MCS was 52·1 (10·6).

12 (75%) patients had at least one TEAE, with ten (63%) having treatment-related TEAEs (table 2). TEAEs reported in two or more patients were injection site reactions (pain, pruritus, erythema, and induration), diarrhoea, fatigue, dizziness, headache, and hypertension. TEAEs in two or more patients considered related or possibly related to treatment were injection site reactions. All TEAEs occurring in two or more patients were mild or moderate in severity, except headache noted as moderate in two patients. There was a serious AE of adenocarcinoma of the pancreas that was considered severe and unrelated

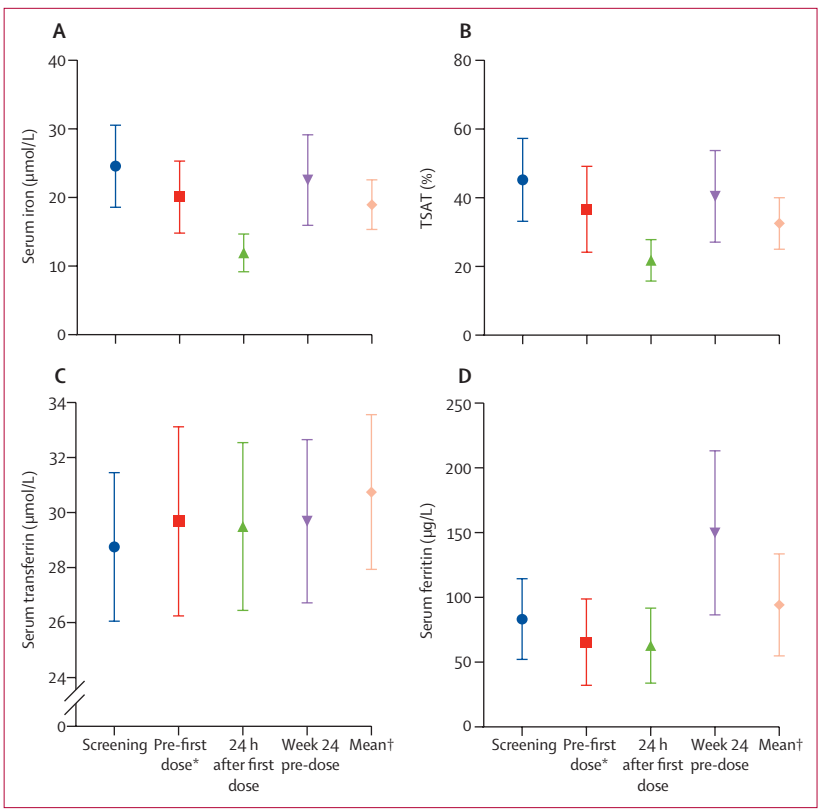

Figure 3: **Effect of rusfertide treatment on iron indices**
Point estimates are mean values and error bars represent 95% CI. (A) Serum iron. (B) TSAT. (C) Serum transferrin. (D) Serum ferritin. TSAT=transferrin iron saturation. *After the pre-treatment phlebotomy. †Over the duration of rusfertide treatment.

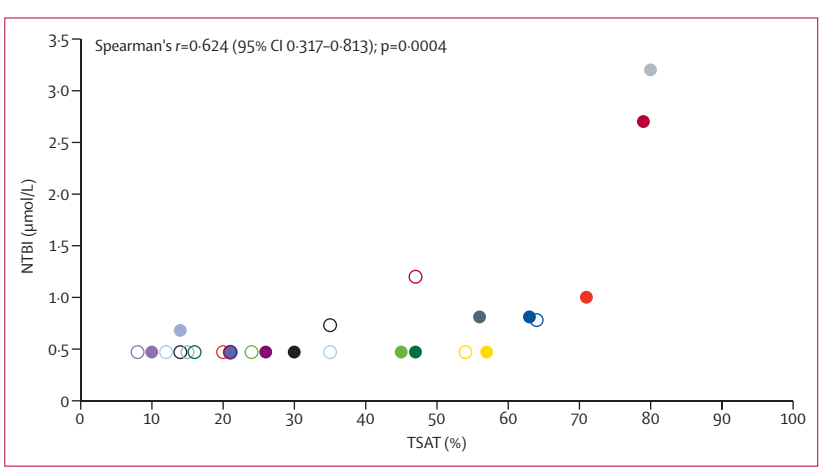

Figure 4: **Relationship between TSAT and NTBI following treatment with rusfertide**
Filled symbols represent paired pre-study measurements. Unfilled symbols represent paired measurements following rusfertide treatment. NTBI=non-transferrin-bound iron. TSAT=transferrin iron saturation.

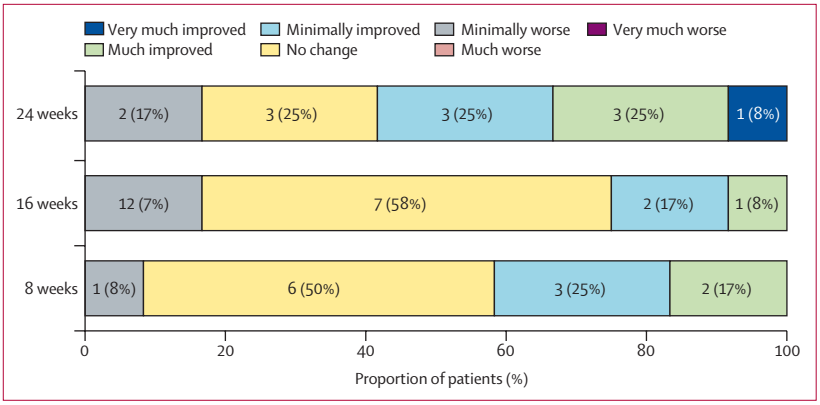

Figure 5: **Patient Global Impression of Change following treatment with rusfertide**
12 patients had Patient Global Impression of Change data for 8 weeks, 16 weeks, and 24 weeks.

	Patients (n=16)
At least one treatment-emergent adverse event	12 (75%)
Serious adverse events	1 (6%)*
Gastrointestinal disorders	
Diarrhoea	2 (13%)
General and administration site conditions	
Injection site pain†	5 (31%)
Injection site pruritus †	3 (19%)
Injection site erythema†	2 (13%)
Injection site induration†	2 (13%)
Fatigue	2 (13%)
Nervous system disorders	
Dizziness	2 (13%)
Headache	2 (13%)
Vascular disorders	
Hypertension	2 (13%)

Data are n (%). Treatment-emergent adverse events reported in two or more patients. *Adenocarcinoma of the pancreas, which was a pre-existing condition diagnosed 21 days after starting rusfertide and was considered not related to the study drug. †Treatment-emergent adverse considered related or possibly related to study drug.

Table 2: **Treatment-emergent adverse events**

to the study drug because a pancreatic mass had been noted on a CT scan done before screening. 21 days after starting treatment with rusfertide, a biopsy confirmed pancreatic adenocarcinoma and the patient subsequently withdrew from the study. Two patients had TEAEs that led to treatment discontinuation. One patient had mild fatigue and injection site swelling and moderate headache that were considered possibly-related TEAEs, and another patient discontinued due to moderate skin rash considered related to treatment.

There were no clinically meaningful changes in clinical laboratory variables, including C-reactive protein, liver enzymes, and haematological variables, such as haematocrit, haemoglobin, reticulocyte haemoglobin content, erythrocytes, white blood cells (leukocytes), or platelets, following rusfertide treatment (appendix p 6). Apart from the expected pharmacodynamic effects of a decrease in serum iron and TSAT values, and an increase in serum ferritin concentration, no consistent treatment effects were noted on serum chemistry parameters. There were no clinically relevant changes in vital signs (systolic and diastolic blood pressure, heart rate, respiratory rate, and body temperature) or on physical examination. There were no clinically notable changes in any of the ECG variables (ventricular rate, PR interval, QRS interval, uncorrected QT interval, RR interval, and Fredericia heart rate-corrected QT interval) with treatment.

Discussion

Hepcidin inhibits both iron absorption from the small intestine and iron release from macrophages. Hepcidin deficiency is a common feature of haemochromatosis.[20,28] Studies in mouse models of haemochromatosis have shown that constitutive expression of hepcidin prevents iron overload.[29] More recently, minihepcidins, hepcidin mimetics, and other agonists have also been shown to prevent iron overload in mouse models of haemochromatosis.[22,24–26] Rusfertide is a potent hepcidin mimetic. To our knowledge, our study is the first proof-of-concept study to investigate the efficacy and safety of rusfertide in patients with HFE-related haemochromatosis. Patients enrolled in the study were in the maintenance phase of treatment with stable regimens of therapeutic phlebotomy. Because their iron overload was well managed, the overall efficacy objective of this study was to investigate whether administration of rusfertide would provide continued disease control in the absence of therapeutic phlebotomy. Following subcutaneous administration, rusfertide resulted in rapid acute decreases in serum iron concentration and TSAT. Serum iron and TSAT at week 24 and the mean over the rusfertide treatment duration were similar to screening values. Similarly, NTBI concentration decreased during treatment with rusfertide. Rusfertide treatment significantly reduced the frequency of phlebotomies compared with the pre-study frequency and only one patient receiving rusfertide underwent a phlebotomy. Correspondingly, the rate of phlebotomy per week also significantly decreased.

The liver is the most affected organ in haemochromatosis and among the first to exhibit iron overload. The baseline mean LIC of 1·4 mg iron per g dry liver weight in our study was similar to the mean LIC of 1·32 mg iron per g dry liver weight reported by a UK Biobank study in which 9108 prospectively identified people aged 40–69 years underwent MRI LIC measurement[30] and is considered within the normal range (<1·8 mg iron per g dry liver weight).[31] In the absence of phlebotomies, nine of 11 patients who remained on rusfertide treatment for 24 weeks showed a decrease in LIC versus baseline. However, LIC

measures total iron and does not reflect iron distribution within the liver. It is possible that potential beneficial changes in iron distribution might not have been captured. Collectively, these clinical observations suggest that rusfertide could be efficacious for disease maintenance and capable of eliminating therapeutic phlebotomies in patients with haemochromatosis.

Treatment with rusfertide resulted in slight improvements in both the mean SF-36 PCS and MCS, but these changes were not clinically meaningful. Of the 12 patients who completed 24 weeks of treatment, ten indicated an improvement or no change in the PGI-C and two indicated minimal worsening. The small number of patients and low burden of symptoms at baseline reduced the prospect that this study would detect improvements in patient-reported outcomes. Rusfertide was generally well tolerated, and all but one TEAE was mild or moderate in severity. Treatment-related TEAEs reported in two or more patients were injection site reactions. No clinically meaningful trends or changes in haematological variables from baseline were noted and there were no haematology findings recorded as TEAEs.

In our study, following an initial decrease 24 h after the first dose, serum ferritin generally increased in concentration with continued rusfertide dosing. These findings are compatible with a pharmacodynamic effect, rather than an overall change in total body iron. A similar observation of dose-related increases in serum ferritin concentration was reported with synthetic human hepcidin, LJPC-401, in healthy participants.[32] Clinically, low ferritin is a marker of iron deficiency; however, high concentrations of ferritin are not synonymous with iron overload.[33] Both the origin and function of serum ferritin are largely unexplored.[34] Rusfertide might decrease duodenal absorption of iron and redistribute serum iron away from the blood and bone marrow compartments, leading to iron retention in splenic macrophages. One speculation is that the increased ferritin concentration following rusfertide reflects a secondary iron restriction response to decreased iron availability. Ferritin is an acute-phase reactant protein that is typically increased in systemic inflammatory conditions. However, we did not see any consistent increases in C-reactive protein, indicating the absence of systemic inflammation as a cause for the increase in ferritin. Rusfertide leads to the redistribution of iron to the reticuloendothelial system, mainly the splenic macrophages. These observations are consistent with findings with PN23114, a rusfertide analogue peptide, in an *HJV*$^{-/-}$ mouse model of haemochromatosis, in which PN23114 treatment resulted in redistribution of excess iron into the spleen and duodenum.[26] Unlike the slight increase in ferritin concentration in patients with HFE-related haemochromatosis in our study, serum ferritin was reduced in the *HJV*$^{-/-}$ mice. By contrast, in a mouse model of polycythaemia vera (*JAK2* Val617Phe mutation), administration of a hepcidin mimetic peptide led to a dose-dependent increase in total iron in the spleen (splenic macrophages) and a corresponding increase in serum ferritin concentration.[35] A recent report noted an increase in serum hepcidin and a positive correlation between serum hepcidin and serum ferritin concentrations in patients with haemochromatosis who had their maintenance phlebotomies interrupted due to COVID-19 pandemic restrictions.[36] Findings of an increase in ferritin concentration with increased hepcidin concentration are similar to our observations with rusfertide, a hepcidin mimetic. Because ferritin is an acute-phase reactant protein and serum ferritin arises from tissue ferritin, we suspect that the increased serum ferritin concentrations noted in our study are a result of iron sequestration in macrophages and ferritin spillover, probably from hepatocytes. Although current treatment guidelines[15] in haemochromatosis focus on maintaining serum ferritin below 50–100 ng/mL, our results, along with the observations of Coutinho and colleagues,[36] suggest that a singular focus on ferritin as a treatment goal should be reconsidered.

TSAT in healthy people without haemochromatosis is approximately 30%. However, in iron overload, TSAT typically exceeds 45%, resulting in circulating NTBI with a high affinity for parenchymal cells. Patients with haemochromatosis exposed to high TSAT might develop worsened clinical symptoms.[37,38] NTBI is normally present in low concentrations but can reach much higher concentrations in patients with haemochromatosis. Le Lan and colleagues[39] reported mean NTBI concentrations of 0·154 µmol/L in healthy controls and 0·761 µmol/L in patients with HFE-related haemochromatosis, which decreased to 0·221 µmol/L following iron depletion. Although the patients enrolled in our study were on maintenance therapeutic phlebotomy, we observed a decrease in NTBI concentration from baseline values following treatment with rusfertide. Similar to published reports,[38] there was a good correlation between TSAT and NTBI concentration. In a small number of patients, we noted a reduction in TSAT and a corresponding reduction in NTBI concentration to less than detectable levels with rusfertide treatment. Monitoring TSAT during therapy might serve as an accessible clinical marker for the presence of NTBI.

Guidelines outline that the goal in the maintenance phase of treatment for haemochromatosis is to maintain serum ferritin at around 50–100 ng/mL.[15] Nine (56%) of the 16 patients enrolled in our study had baseline serum ferritin concentrations of more than 50 ng/mL, suggesting that physicians also incorporate medical judgement in determining the need for phlebotomy. During this clinical trial with rusfertide, the protocol provided guidance as to when investigators should consider a phlebotomy for a patient based on the serum ferritin and TSAT levels at screening. We considered

both serum ferritin and TSAT because previous reports with hepcidin have indicated a pharmacodynamic increase in serum ferritin concentration following administration of hepcidin.[32] Although the use of serum ferritin concentration along with TSAT in this trial might have differed from criteria that physicians could have used pre-study in deciding when to phlebotomise, we believe that the use of both serum ferritin and TSAT was appropriate because they provided standard criteria for all investigators and incorporated key clinical attributes of the disease that could be easily monitored.

Therapeutic phlebotomies are the current mainstay of treatment for patients with HFE-related haemochromatosis. Although phlebotomy is inexpensive and relatively easy to do, up to 100 procedures might be required in patients with a high initial iron burden to achieve initial normalisation before maintenance phlebotomy is implemented.[40,41] In addition, an estimated 15–25% of patients with haemochromatosis undergoing phlebotomy expressed a negative opinion about the procedure, focused on venous access, travel time, waiting, and the duration of the procedure.[16,42,43] Treatment with rusfertide in our study allowed the reduction and maintenance of serum iron and TSAT, similarly to phlebotomy. A pharmacological therapeutic, such as rusfertide, which can be titrated on the basis of the values of common clinical markers, such as TSAT or serum iron, could allow better disease control. For patients who have a high phlebotomy burden or are unable to receive or are intolerant of phlebotomies, a therapeutic option such as rusfertide might be well suited.

The limitations of this proof-of-concept study include the open-label design, absence of a placebo control, the exploratory nature of observations in a small group of patients on maintenance phlebotomy, and the allowance for within-patient dose changes based on iron indices. Regression to the mean and variability in time trends are also potential limitations of trials with a before-and-after design, including potentially differing criteria for phlebotomy prestudy and during the study. Proof-of-concept studies present statistical challenges given their small sample sizes. However, our results provide valuable insight for the design of future studies in subgroups of patients with haemochromatosis who are burdened by the need for recurrent phlebotomies.

In conclusion, to our knowledge, this clinical study is the first to show the safety and efficacy of rusfertide, a hepcidin mimetic, in patients with HFE-related haemochromatosis. Rusfertide significantly reduced the mean number of phlebotomies compared with the number before treatment. The results of our study indicate that rusfertide might be an alternative for managing iron accumulation in patients who are unwilling or unable to undergo therapeutic phlebotomy. Larger studies should be done to fully define the role of rusfertide in the treatment of selected patients with haemochromatosis and to further characterise its safety and tolerability.

Contributors

KVK, NBM, KP, FHV, and SG were involved in the study concept and design. KVK, NBM, FHV, and SG were involved in the collection and analysis of data. KVK, KP, JMV, and CF were responsible for recruiting patients and collecting data. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication. KVK, NBM, FHV, and SG accessed and verified the data (KVK is an academician).

Declaration of interests

KVK, KP, JMV, and CF were investigators in the trial and received grants from the study sponsor paid to their institutions to conduct the study. KVK has received royalties from UptoDate; has received consulting fees from CymaBay, Enanta, Genfit, HighTide, Inipharm, Intercept, Ipsen, Madrigal, Mirum, NGM, Pliant, Pfizer, Protagonist, 89bio, and Zydus; has received honoraria from AbbVie, Gilead, Intercept, and the US Department of Justice; has received payment as a participant on a data safety monitoring board or advisory board from Medpace, Labcorp, Worldwide Clinical Trials, and CTI; has stock or stock options with Inipharm; and has received equipment material, drugs, medical writing, gifts, or other services from Velacur. In addition, his institutions have received grants from Boston, Corcept, CymaBay, Intercept, Ipsen, Janssen, Madrigal, Mirum, Novo Nordisk, NGM, Pfizer, Pliant, Terns, Viking, 89bio, and Zydus. JMV has received payment as a participant on a data safety monitoring board or advisory board from the US National Institutes of Health, the US National Institute of Diabetes and Digestive and Kidney Diseases, and Fractyl and has stock or stock options in Athenex. NBM, FHV, and SG are employees or consultants of Protagonist Therapeutics and have received payments for employment, consulting, or travel, and have stock or stock options in Protagonist Therapeutics.

Data sharing

Data will be shared with bona fide researchers, upon reasonable request, who submit a research proposal to the corresponding author approved by an independent review board. Data will be made available after research completion and approval of the product and product use in the EU and the USA.

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