ORIGINAL ARTICLE

Rusfertide, a Hepcidin Mimetic, for Control of Erythrocytosis in Polycythemia Vera

M. Kremyanskaya, A.T. Kuykendall, N. Pemmaraju, E.K. Ritchie, J. Gotlib, A. Gerds, J. Palmer, K. Pettit, U.K. Nath, A. Yacoub, A. Molina, S.R. Saks, N.B. Modi, F.H. Valone, S. Khanna, S. Gupta, S. Verstovsek, Y.Z. Ginzburg, and R. Hoffman, for the REVIVE Trial Investigators*

ABSTRACT

BACKGROUND

Polycythemia vera is a chronic myeloproliferative neoplasm characterized by erythrocytosis. Rusfertide, an injectable peptide mimetic of the master iron regulatory hormone hepcidin, restricts the availability of iron for erythropoiesis. The safety and efficacy of rusfertide in patients with phlebotomy-dependent polycythemia vera are unknown.

METHODS

In part 1 of the international, phase 2 REVIVE trial, we enrolled patients in a 28-week dose-finding assessment of rusfertide. Part 2 was a double-blind, randomized withdrawal period in which we assigned patients, in a 1:1 ratio, to receive rusfertide or placebo for 12 weeks. The primary efficacy end point was a response, defined by hematocrit control, absence of phlebotomy, and completion of the trial regimen during part 2. Patient-reported outcomes were assessed by means of the modified Myeloproliferative Neoplasm Symptom Assessment Form (MPN-SAF) patient diary (scores range from 0 to 10, with higher scores indicating greater severity of symptoms).

RESULTS

Seventy patients were enrolled in part 1 of the trial, and 59 were assigned to receive rusfertide (30 patients) or placebo (29 patients) in part 2. The estimated mean (±SD) number of phlebotomies per year was 8.7±2.9 during the 28 weeks before the first dose of rusfertide and 0.6±1.0 during part 1 (estimated difference, 8.1 phlebotomies per year). The mean maximum hematocrit was 44.5±2.2% during part 1 as compared with 50.0±5.8% during the 28 weeks before the first dose of rusfertide. During part 2, a response was observed in 60% of the patients who received rusfertide as compared with 17% of those who received placebo (P=0.002). Between baseline and the end of part 1, rusfertide treatment was associated with a decrease in individual symptom scores on the MPN-SAF in patients with moderate or severe symptoms at baseline. During parts 1 and 2, grade 3 adverse events occurred in 13% of the patients, and none of the patients had a grade 4 or 5 event. Injection-site reactions of grade 1 or 2 in severity were common.

CONCLUSIONS

In patients with polycythemia vera, rusfertide treatment was associated with a mean hematocrit of less than 45% during the 28-week dose-finding period, and the percentage of patients with a response during the 12-week randomized withdrawal period was greater with rusfertide than with placebo. (Funded by Protagonist Therapeutics; REVIVE ClinicalTrials.gov number, NCT04057040.)

The authors' full names, academic degrees, and affiliations are listed in the Appendix. Dr. Kremyanskaya can be contacted at marina.kremyanskaya@mssm.edu or at the Division of Hematology and Medical Oncology, Tisch Cancer Institute, Icahn School of Medicine at Mount Sinai, 1 Gustave L. Levy Pl., New York, NY 10029.

*A list of the investigators in the REVIVE trial is provided in the Supplementary Appendix, available at NEJM.org.

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A Quick Take is available at NEJM.org

Polycythemia vera is a myeloproliferative neoplasm that is characterized by erythrocytosis and variable increases in leukocyte and platelet counts. Most patients with polycythemia vera have a constitutively activating somatic mutation in *JAK2*, which leads to the overproduction of terminally differentiated hematopoietic cells.[1-5] The incidence of thromboembolic events is higher among patients with polycythemia vera than among persons without polycythemia vera — a finding that has primarily been attributed to erythrocytosis, although some trials have suggested that leukocytosis, thrombocytosis, or both could also be important risk factors.[6] Polycythemia vera is accompanied by systemic symptoms, including pruritus, night sweats, difficulty concentrating, and fatigue.[7,8]

The therapeutic approach in polycythemia vera is to reduce the risk of thromboembolic events by maintaining a hematocrit of less than 45%.[9-11] Current treatments for polycythemia vera are based on a risk assessment for thrombosis and include aspirin and phlebotomy in low-risk patients and the addition of cytoreductive agents, such as hydroxyurea, ruxolitinib, and interferon alfa, in high-risk patients (those who are ≥60 years old, had previous thrombosis, or both).[12-15] Real-world studies have shown that currently available treatments do not effectively maintain a hematocrit of less than 45% in a large percentage of patients with polycythemia vera, leaving many patients at high risk for complications.[16,17] In a study that included 2510 patients with polycythemia vera in academic and community centers, 47.7% of the patients had a hematocrit of more than 45% at study entry.[17]

Hepcidin, a peptide hormone produced in the liver, is a master regulator of iron trafficking and is up-regulated by circulating iron levels and inflammatory cytokines and down-regulated by bone marrow erythroid hyperplasia.[18-20] Hepcidin binds to ferroportin, which blocks the export of intracellular iron to the blood and leads to reduced levels of serum iron and transferrin saturation. Reduced export of iron results in functional iron deficiency and decreased erythropoiesis.[21] Preclinical models suggest that increasing the hepcidin activity in patients with polycythemia vera could be effective in controlling erythrocytosis and might alleviate symptoms.[22-25]

Rusfertide is an injectable, peptide hepcidin–mimetic compound with a mechanism of action similar to that of endogenous hepcidin. In healthy volunteers, rusfertide decreased serum iron levels in a dose-dependent fashion,[26] which suggests that rusfertide may decrease erythropoiesis in patients with polycythemia vera. We performed the phase 2 REVIVE trial to evaluate the safety and efficacy of rusfertide in patients with phlebotomy-dependent polycythemia vera.

METHODS

TRIAL DESIGN AND OVERSIGHT

REVIVE is an ongoing, international, three-part, phase 2 trial conducted in 16 centers in the United States and India. An overview of the trial design is provided in Figure S1 in the Supplementary Appendix, available with the full text of this article at NEJM.org. Part 1 of the trial was a 28-week, open-label, dose-finding period (baseline to week 29) in which rusfertide was added to the patient's ongoing therapy (phlebotomy alone or cytoreductive therapy with supplemental phlebotomy). Rusfertide was administered subcutaneously weekly, and the dose, which ranged from 10 to 120 mg, was adjusted to maintain a hematocrit of less than 45%. Part 2 was a 12-week, double-blind, randomized withdrawal period (weeks 29 to 41) in which patients were assigned, in a 1:1 ratio, to receive rusfertide or placebo. Part 2 was unblinded in March 2023 and is the focus of the current article. Part 3 is an ongoing open-label extension period (week 41 up to year 3) in which all the patients are receiving rusfertide.

Adverse events were monitored throughout the trial and were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 5.0.[27] Laboratory assessments were performed according to the trial protocol (available at NEJM.org). An independent safety monitoring committee routinely reviewed safety data.

The trial was sponsored and designed by Protagonist Therapeutics in collaboration with the investigators. Data were collected by the investigators, analyzed by statisticians employed by the sponsor, and interpreted by all the authors. All the authors had full access to the data and were responsible for content and editorial decisions related to the preparation of the manuscript. The sponsor provided and funded editorial support for the preparation of the manuscript. All the authors vouch for the accuracy and completeness

of the data and for the fidelity of the trial to the protocol. Additional details of the trial design and oversight are provided in the Supplementary Appendix.

During the trial, the Food and Drug Administration placed the trial on clinical hold so that all the patients and investigators could be informed about a potential tumorigenicity signal after the observation of skin lesions — predominantly papillomas — during a 26-week carcinogenicity study in a rasH2 transgenic mouse model. The clinical hold required the updating of rusfertide trial documents with information about nonclinical results and clinical cancer events. The hold was lifted after 3 weeks, and the administration of rusfertide resumed after the investigators received institutional approval to continue the trial.

PATIENTS AND ELIGIBILITY

Adults (≥18 years of age) were eligible for the trial if they met the revised 2016 World Health Organization criteria for the diagnosis of polycythemia vera and had phlebotomy-dependent disease, defined as at least three phlebotomies during the 28 weeks before the first dose of rusfertide in part 1 of the trial, with the most recent phlebotomy having occurred within 12 weeks before screening. Patients who had been receiving a stable or decreasing dose of hydroxyurea, interferon alfa, or ruxolitinib for at least 8 weeks before the first dose of rusfertide in part 1 and for whom the dose of the cytoreductive agent was not anticipated to change during the trial were allowed to participate in the trial and to continue cytoreductive treatment.

END POINTS

The primary efficacy end point was a response during the randomized withdrawal period (part 2). Patients were considered to have had a response if they had hematocrit control, did not undergo phlebotomy, and completed the 12-week trial regimen during part 2. Hematocrit control was defined as not meeting any of the criteria for phlebotomy eligibility, which included a hematocrit of at least 45% that was at least 3 percentage points higher than the hematocrit at week 29 (before randomization), a hematocrit of more than 48%, or a hematocrit that was at least 5 percentage points higher than the hematocrit at week 29 (before randomization).

Secondary end points in part 1 of the trial included the change in the phlebotomy rate (number of phlebotomies per year) between the 28-week period before the first dose of rusfertide and part 1, the change in the phlebotomy rate between weeks 16 to 28 before the first dose of rusfertide and the efficacy evaluation period of part 1 (weeks 17 to 29), and a response, defined as the absence of phlebotomy eligibility (a hematocrit of ≥45% that was ≥3 percentage points higher than the level at baseline or a hematocrit of >48%) during the efficacy evaluation period of part 1. Phlebotomy rates are presented as mean values

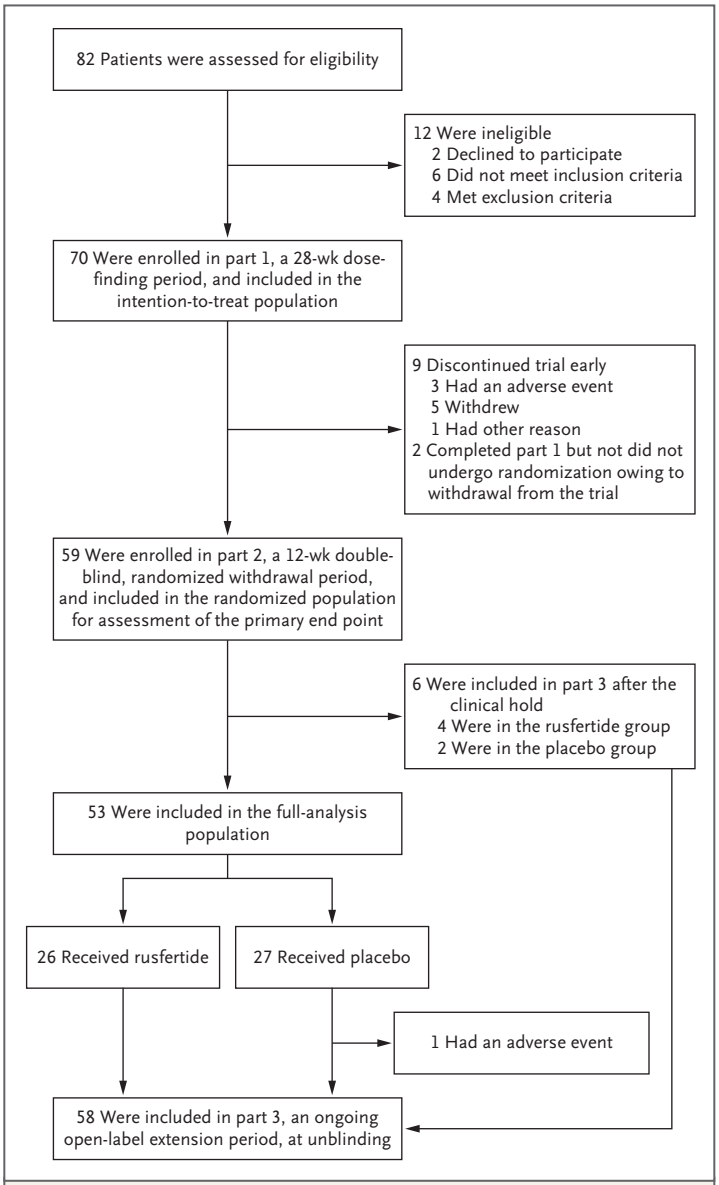


Figure 1. Trial Screening, Enrollment, Disposition, and Randomization.

with standard deviations. Secondary end points in part 2 included the change in the hematocrit from baseline to week 41.

We also assessed the change from baseline to week 29 in individual symptom scores and the total symptom score on the modified Myeloproliferative Neoplasm Symptom Assessment Form (MPN-SAF) patient diary (Fig. S2).[28] The MPN-SAF was used to assess the severity of 10 symptoms related to polycythemia vera on a scale from 0 (absent) to 10 (worst). The MPN-SAF has been validated for the evaluation of symptoms that occur in patients with polycythemia vera. The changes in individual symptom scores on the MPN-SAF from baseline to prespecified time points were assessed in patients with moderate symptoms (score, 4 to 6) or severe symptoms (score, 7 to 10) at baseline. A mean difference of 0.92 points in individual symptom scores is considered to be meaningful.[29]

STATISTICAL ANALYSIS

Efficacy in part 1 of the trial was assessed in the intention-to-treat population, which included all the patients who received rusfertide at any dose. The analysis of the primary efficacy end point was performed in the randomized population, which included all the patients who underwent randomization in part 2. As prespecified in the statistical analysis plan, primary efficacy during part

Table 1. Demographic and Clinical Characteristics of the Patients at Baseline.*

Characteristic	Part 1 (N=70)	Part 2	
		Placebo (N=29)	Rusfertide (N=30)
Age — yr	57.3±12.2	60.6±11.3	54.2±13.0
Sex — no. (%)			
Male	49 (70)	18 (62)	24 (80)
Female	21 (30)	11 (38)	6 (20)
Race or ethnic group — no. (%)†			
White	57 (81)	25 (86)	28 (93)
Black	2 (3)	0	1 (3)
Asian	5 (7)	2 (7)	1 (3)
American Indian or Alaska Native	2 (3)	1 (3)	0
Other or multiple	2 (3)	1 (3)	0
Not reported	2 (3)	0	0
Body-mass index‡	29.6±5.4	29.9±5.7	29.3±5.3
Median serum ferritin level (range) — μg/liter	13.0 (3–255)	13.5 (4–255)	11.5 (3–51)
Age at diagnosis of polycythemia vera — yr	52.3±13.5	55.8±12.1	49.9±12.4
Duration of polycythemia vera — yr	5.1±6.2	4.9±4.6	4.2±5.4
Phlebotomy history§			
No. of phlebotomies	4.7±1.6	4.8±1.6	4.7±1.6
Underwent ≥5 phlebotomies — no. (%)	31 (44)	14 (48)	13 (43)
Risk category — no. (%)¶			
High-risk disease			
Overall	40 (57)	18 (62)	13 (43)
Age of ≥60 yr only	26 (37)	13 (45)	8 (27)
Previous thrombotic event only	7 (10)	1 (3)	3 (10)
Age of ≥60 yr and previous thrombotic event	7 (10)	4 (14)	2 (7)
Low-risk disease	30 (43)	11 (38)	17 (57)

Table 1. (Continued.)

Characteristic	Part 1 (N=70)	Part 2	
		Placebo (N=29)	Rusfertide (N=30)
Concurrent therapy — no. (%)			
Phlebotomy alone			
Overall	37 (53)	12 (41)	20 (67)
High-risk disease	19 (27)	8 (28)	6 (20)
Low-risk disease	18 (26)	4 (14)	14 (47)
Cytoreductive therapy with supplemental phlebotomy			
Overall	33 (47)	17 (59)	10 (33)
Risk category			
High-risk disease	21 (30)	10 (34)	7 (23)
Low-risk disease	12 (17)	7 (24)	3 (10)
Agent			
Hydroxyurea only	18 (26)	10 (34)	4 (13)
Interferon alfa only	8 (11)	5 (17)	2 (7)
Ruxolitinib only	5 (7)	2 (7)	3 (10)
Multiple agents	2 (3)	0	1 (3)

* Plus–minus values are means ±SD. Percentage may not total 100 because of rounding. Characteristics at baseline are provided for the intention-to-treat population, which included all the patients who received rusfertide at any dose in part 1 of the trial (dose-finding period; baseline to week 29), and for the randomized population, which included all the patients who were randomly assigned to receive rusfertide or placebo in part 2 of the trial (randomized withdrawal period; weeks 29 to 41). Six patients in the randomized population discontinued the trial regimen because of the clinical hold; the patients were rolled over into the open-label extension period of the trial (part 3; week 41 up to year 3) after the clinical hold was lifted and were considered as not having had a response.

† Race and ethnic group were reported by the patients.

‡ The body-mass index is the weight in kilograms divided by the square of the height in meters. Data are missing for 2 patients, both of whom were randomly assigned to the placebo group in part 2.

§ Data on phlebotomies that occurred during the 28-week period before the start of rusfertide therapy are provided.

¶ Patients who are 60 years of age or older, have had a thromboembolic event, or both are considered to have high-risk disease, and patients who are younger than 60 years of age and have not had a thromboembolic event are considered to have low-risk disease.[10]

2 was assessed in the full analysis population, which excluded the 6 patients who discontinued the trial regimen in part 2 because of the clinical hold (see the Supplementary Appendix).

Assuming that a response would occur in approximately 32% of patients in the placebo group and approximately 80% of patients in the rusfertide group during part 2 of the trial, we estimated that the enrollment of 50 patients would provide a power of at least 90% to detect a between-group difference in the percentage of patients with a response, at a two-sided alpha level of 0.05. The primary efficacy end point was analyzed with the use of Fisher's exact test (two-sided).

Data for continuous end points, such as the phlebotomy rate during the 28 weeks before the first dose of rusfertide and the phlebotomy rate after the first dose of rusfertide, are presented for part 1; the data exclude the clinical hold duration. We performed time-to-event analyses using the Kaplan–Meier method to estimate the median times to loss of response, phlebotomy eligibility, and a hematocrit of at least 45% in part 2.

Missing primary end-point data were imputed with the use of various statistical techniques to evaluate the robustness of the primary end-point analysis. For other end points, no imputation of missing data was performed, and data are presented as mean values with standard errors of the mean.

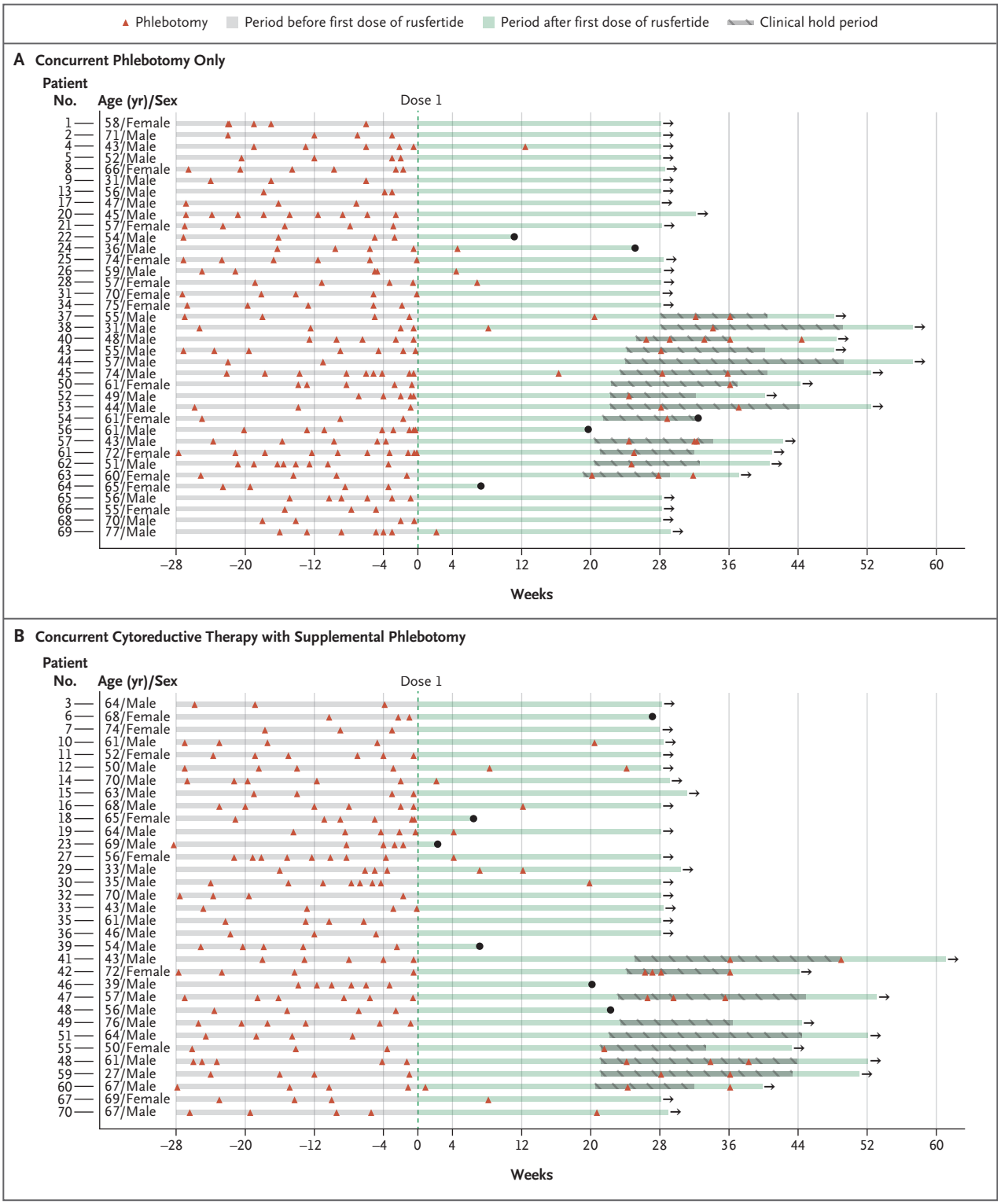


Figure 2 (facing page). Phlebotomies before and after the First Dose of Rusfertide.

Panels A and B show swimmer plots of phlebotomies that were performed before the first dose of rusfertide and those performed during part 1 of the trial in the intention-to-treat population, according to concurrent treatment for polycythemia vera. The number of phlebotomies increased during the clinical hold, when patients were not receiving rusfertide therapy. The dots indicate the time at which patients discontinued the trial, and the arrows indicate that participation in the trial is ongoing.

RESULTS

PATIENTS

Between October 2019 and March 2022, we screened 82 patients; 70 were enrolled in part 1 of the trial (intention-to-treat population), and 59 were randomly assigned to receive rusfertide or placebo in part 2 of the trial (randomized population) (Fig. 1). Of the 11 patients who did not undergo randomization, 3 discontinued the trial because of adverse events, and 8 discontinued the trial for other reasons. The primary end-point analysis was performed in the randomized population. The administration of the trial regimen was stopped because of the clinical hold in 6 patients in the randomized population.

The demographic and disease characteristics at baseline in the intention-to-treat population are provided in Table 1. Concurrent treatment for polycythemia vera included phlebotomy alone in 37 patients (53%) and cytoreductive therapy with supplemental phlebotomy in 33 patients (47%). Thirty patients (43%) had low-risk disease, and 40 patients (57%) had high-risk disease.[10] More than 30% of the patients had had polycythemia vera for more than 5 years. The mean (±SD) number of phlebotomies during the 28 weeks before the first dose of rusfertide was high (4.7±1.6). At the time of unblinding, rusfertide therapy had been received by 52 patients (74%) for at least 1 year, by 32 (46%) for at least 1.5 years, and by 10 (14%) for at least 2 years.

Demographic and disease characteristics at baseline in the randomized population are provided in Table 1. Characteristics of the trial regimens during parts 1 and 2 are provided in Table S2.

EFFICACY

Part 1 of the Trial

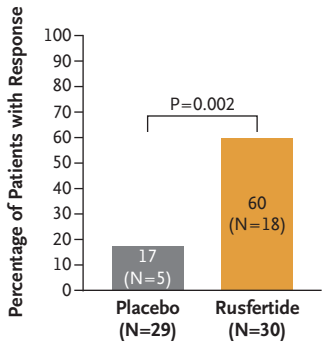
The use of phlebotomy decreased or ceased in all the patients after the initiation of rusfertide treatment (Fig. 2). The estimated mean phlebotomy rate was 8.7±2.9 during the 28 weeks before the first dose of rusfertide and 0.6±1.0 during part 1 of the trial (baseline to week 29), with an estimated difference of 8.1 phlebotomies per year (Table S3). During the efficacy evaluation period (weeks 17 to 29), the estimated mean phlebotomy rate was low (Table S3), and a response occurred in 58 of 70 patients (83%) (Table S4). The estimated mean phlebotomy rate during part 1 was low regardless of polycythemia vera risk category (high or low) or concurrent treatment (phlebotomy alone or cytoreductive therapy with supplemental phlebotomy) (Table S5). The mean maximum hematocrit was lower during part 1 (44.5±2.2%) than during the 28 weeks before the first dose of rusfertide (50.0±5.8%).

Part 2 of the Trial

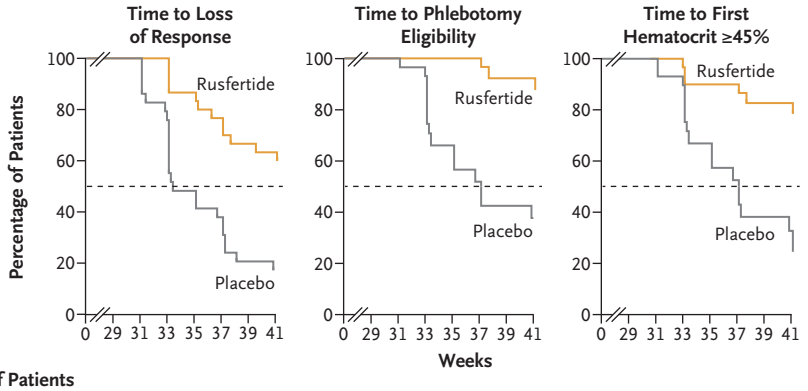
The primary end-point analysis in part 2 of the trial (weeks 29 to 41) included 59 patients (randomized population): 30 in the rusfertide group and 29 in the placebo group. A response occurred in 60% of the patients in the rusfertide group and in 17% of those in the placebo group (P=0.002) (Fig. 3A). A sensitivity analysis of missing hematocrit data in the randomized population was performed with the use of multiple imputation with a delta adjustment, and the results supported the robustness of the primary end-point analysis (Table S7). In both the rusfertide group and the placebo group, the percentage of patients with a response was similar in the full analysis population (Fig. S3) and the randomized population (Fig. 3A).

Criteria for phlebotomy eligibility were met by 3 of 30 patients (10%) in the rusfertide group and by 15 of 29 patients (52%) in the placebo group. Reasons for nonresponse in the patients who did not meet the criteria for phlebotomy eligibility are listed in Table S8. Four of the patients in the rusfertide group and 2 of those in the placebo group discontinued the trial regimen because of the clinical hold and were considered not to have had a response. A total of 28 patients (93%) in the rusfertide group and 14 patients (48%) in the placebo group did not undergo phlebotomy

A Primary End-Point Analysis



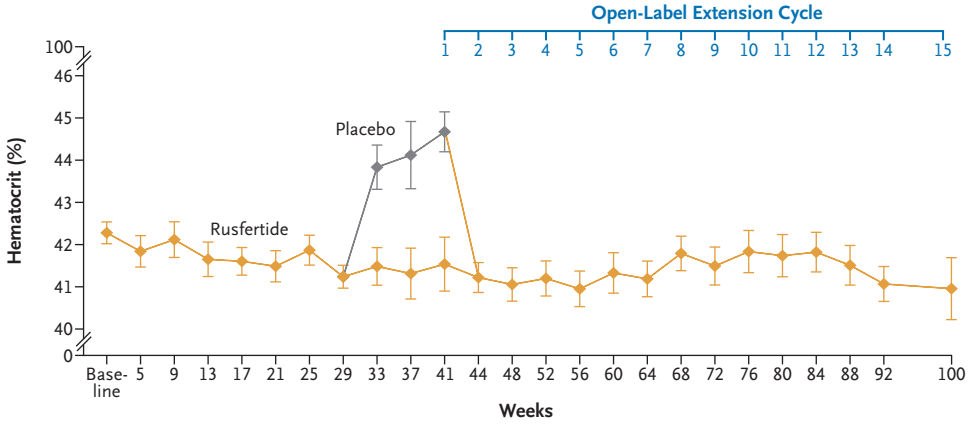
B Time-to-Event Analysis



No. of Patients

Rusfertide	30	30	26	25	21	20	15
Placebo	29	25	22	12	9	6	5
Rusfertide	30	30	30	30	22	20	15
Placebo	29	28	25	12	9	9	5
Rusfertide	30	30	29	27	22	20	15
Placebo	29	27	25	12	9	7	3

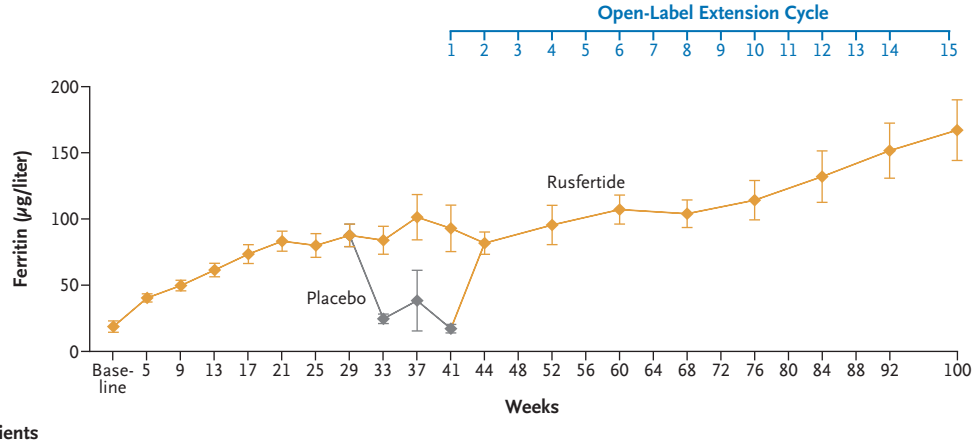
C Hematocrits over Time



No. of Patients

Rusfertide	58	60	59	58	58	55	41	59	30	22	19	56	55	49	47	49	47	43	39	38	32	28	25	24	16
Placebo									27	15	7														

D Ferritin Levels over Time



No. of Patients

Rusfertide	58	52	56	55	53	54	35	56	30	20	18	55		51		48		48		36		29		21	18
Placebo									26	14	7														

Figure 3 (facing page). Efficacy, Time to Treatment Failure, Hematocrit, and Ferritin Level (Randomized Population).

A total of 59 patients were randomly assigned to receive rusfertide or placebo in part 2 of the trial (weeks 29 to 41) and were included in the randomized population. The clinical hold interrupted the trial regimen in 6 patients after randomization; the patients were included in part 3 after the clinical hold was lifted and were considered as not having had a response during part 2. Panel A shows the percentage of patients with a response, defined as hematocrit control, absence of phlebotomy, and completion of the trial regimen during part 2 (primary efficacy end point). Hematocrit control was defined as not meeting any of the criteria for phlebotomy eligibility, which included a hematocrit of at least 45% that was at least 3 percentage points higher than the hematocrit at week 29 (before randomization), a hematocrit of more than 48%, or a hematocrit that was at least 5 percentage points higher than the hematocrit at week 29 (before randomization). The P value was calculated with the use of Fisher's exact test (two-sided). Panel B shows Kaplan–Meier time-to-event analyses of the times until the loss of response, phlebotomy eligibility, and a first hematocrit of at least 45% during part 2. The dashed lines indicate the median time to the event (50th percentile). Panels C and D show mean hematocrits and mean ferritin levels, respectively, during part 1 (baseline to week 29), part 2, and part 3 (week 41 up to year 2 part 3 is ongoing). The open-label extension period is composed of 18 cycles; cycles 1 through 13 are 4 weeks long, and cycles 14 through 18 are 8 weeks long. Blood samples for the assessments of hematocrits and ferritin levels were collected before the administration of rusfertide or placebo. I bars indicate the standard error of the mean.

during part 2 of the trial (Table S9). At the end (completion or discontinuation) of part 2, the mean absolute change from baseline in the hematocrit was 0.3±3.1 percentage points in the rusfertide group and 2.0±2.6 percentage points in the placebo group.

The percentages of patients in the randomized population with a response during part 2 are presented for the rusfertide and placebo groups according to risk (high or low) and concurrent treatment (phlebotomy alone or cytoreductive therapy with supplemental phlebotomy) in Figure S4 and Table S10. Findings in the full analysis population were similar to those in the randomized population (Fig. S5).

The median times to loss of response, phlebotomy eligibility, and a first hematocrit of at least 45% were not reached in the rusfertide group during part 2 (Fig. 3B). In the placebo group, the median time from the start of part 2 until the loss of a response was 4.4 weeks, and the median times from the start of part 2 until phlebotomy eligibility and a first hematocrit of at least 45% were both 8.1 weeks. Results of the time-to-event analyses in the full analysis population were similar to those in the randomized population (Fig. S6).

ADDITIONAL EFFECTS OF RUSFERTIDE

During rusfertide treatment, the mean hematocrit was consistently lower than 45% (Fig. 3C), and the mean erythrocyte count was less than that at baseline (Fig. S7). The mean hematocrit and the mean erythrocyte count increased in the placebo group during part 2. Changes in the hematocrit and the erythrocyte count after the withdrawal of rusfertide therapy because of the clinical hold were similar to changes in the hematocrit and erythrocyte count in the patients who were assigned to the placebo group in part 2 (Fig. S8). The mean leukocyte count was stable during part 1 (Fig. S9). The mean platelet count increased by approximately 30% between baseline and week 5 after the initiation of rusfertide therapy and remained stable to week 41 (Fig. S10).

The median serum ferritin level at baseline (13.0 μg per liter; range, 3 to 255) was consistent with systemic iron deficiency (Table 1 and Table S11). The mean serum ferritin level increased during rusfertide treatment (Fig. 3D). The mean corpuscular volume (Fig. S11), mean transferrin saturation level (Fig. S12A), and mean serum iron level (Fig. S13) increased slightly during rusfertide treatment but remained low. The increase in the mean transferrin saturation level between baseline and the latest assessment in part 3 was higher among patients with a baseline ferritin level of less than 20 μg per liter than among those with a baseline ferritin level of at least 20 μg per liter. (Fig. S12B).

PATIENT-REPORTED OUTCOMES

At baseline, the mean total symptom score on the MPN-SAF was low (15.6±15.3). In part 1, rusfertide treatment was associated with a lower severity of disease-related symptoms, which included fatigue, early satiety, night sweats, problems with concentration, inactivity, and itching, in patients with moderate symptoms (MPN-SAF

score, 4 to 6) or severe symptoms (MPN-SAF score, 7 to 10) at baseline (Fig. S14). A comparison of the trial groups with respect to symptom scores in part 2 was not possible because of the early nonresponse to placebo in most of the patients in the placebo group.

SAFETY

Adverse events that occurred during parts 1 and 2 of the trial are shown in Table 2. The incidence of adverse events among patients who received concurrent cytoreductive therapy was similar to that among patients who did not receive concurrent cytoreductive therapy (Table S12). All the patients had at least one adverse event of grade 1 or 2 in severity. Ten grade 3 adverse events occurred in nine patients (13%); one patient each had myocardial infarction, fatigue, an increased platelet count, peripheral sensory neuropathy, syncope, squamous-cell carcinoma, basal-cell carcinoma, and hypertension, and one patient had both acute myeloid leukemia and squamous-cell carcinoma. No grade 4 or 5 adverse events were observed. Injection-site reactions were the most common adverse events (Table 2 and Table S13). All injection-site reactions were grade 1 or 2 in severity, were localized, and decreased in incidence with continued treatment. Four patients withdrew because of an adverse event (asymptomatic thrombocytosis, nephrolithiasis, injection-site erythema, and acute myeloid leukemia). Serious adverse events were consistent with polycythemia vera and underlying coexisting conditions (Table S14). No clinically significant abnormal laboratory findings were reported.

Grade 2 superficial vein thrombosis occurred in one patient with high-risk disease. Myocardial infarction occurred in another patient with high-risk disease; atherosclerosis or clinically significant stenosis were not identified on coronary

Table 2. Adverse Events during Parts 1 and 2 of the Trial.*

Event	Part 1 (N = 70)		Part 2				Overall (N = 70)	
			Placebo (N = 29)		Rusfertide (N = 30)			
	Any	Grade 3	Any	Grade 3	Any	Grade 3	Any	Grade 3
	number of patients (percent)							
Any adverse event	69 (99)	7 (10)	16 (55)	2 (7)	24 (80)	0	70 (100)	9 (13)
Injection-site reaction†	60 (86)	0	3 (10)	0	13 (43)	0	60 (86)	0
Fatigue	16 (23)	1 (1)	1 (3)	0	1 (3)	0	18 (26)	1 (1)
Pruritus	14 (20)	0	3 (10)	0	2 (7)	0	17 (24)	0
Nausea	11 (16)	0	2 (7)	0	1 (3)	0	14 (20)	0
Arthralgia	13 (19)	0	0	0	0	0	13 (19)	0
Headache	11 (16)	0	2 (7)	0	0	0	13 (19)	0
Anemia	12 (17)	0	0	0	0	0	12 (17)	0
Dizziness	9 (13)	0	0	0	0	0	9 (13)	0
Dyspnea	6 (9)	0	2 (7)	0	1 (3)	0	9 (13)	0
Hyperuricemia	6 (9)	0	1 (3)	0	2 (7)	0	8 (11)	0
Diarrhea	7 (10)	0	1 (3)	0	0	0	7 (10)	0
Insomnia	7 (10)	0	0	0	0	0	7 (10)	0
Myalgia	4 (6)	0	1 (3)	0	2 (7)	0	7 (10)	0
Paresthesia	4 (6)	0	1 (3)	0	2 (7)	0	7 (10)	0

* Adverse events that occurred in at least 10% of the patients in the intention-to-treat population during parts 1 and 2 are presented according to *Medical Dictionary for Regulatory Activities*, version 25.0, preferred term. Patients with multiple occurrences of the same event are counted only once. No grade 4 or 5 events were reported.
† Injection-site reactions that occurred in at least 10% of the patients are listed in Table S13.

angiography, and myocardial infarction was attributed to coronary artery spasm unrelated to rusfertide treatment. Of the 85% of patients with a thromboembolic event before the first dose of rusfertide, none had a thromboembolic event during parts 1 or 2 of the trial.

Cancer had previously occurred in 19 of the 70 patients (27%), with skin cancer having occurred in 10 (14%). Five patients (7%) had a second malignant condition — in situ or stage 1 skin cancer — diagnosed after the initiation of rusfertide therapy (Table S15). In 4 of these patients, risk factors for the second malignant condition included previous cancer and exposure to either hydroxyurea or ruxolitinib. The fifth patient had basal-cell carcinoma that was present before the first dose of rusfertide but diagnosed after the treatment began. The investigators did not consider any malignant events to be related to rusfertide therapy.

DISCUSSION

This trial shows that rusfertide therapy controlled erythrocytosis, maintained a hematocrit of less than 45%, and reduced or eliminated the use of phlebotomy in patients with polycythemia vera. These responses were maintained, with some of the patients not having undergone phlebotomy during more than 2.5 years of follow-up. Although maintenance of a hematocrit of less than 45% reduces the risk of thrombotic events,[11] additional trials and longer follow-up will be needed to examine whether rusfertide therapy affects the incidence of thromboembolic events. In contrast to its effects on erythrocytosis, rusfertide therapy was associated with a modest increase in platelet counts and did not affect leukocyte counts.

Preclinical studies in mice with polycythemia vera showed that hepcidin mimetics are effective at controlling erythrocytosis without evidence of tissue iron depletion.[22,30] Consistent with the mechanism of action of hepcidin, rusfertide treatment also led to increased serum ferritin levels, which indicates a redistribution of systemic iron stores and a partial shift from systemic to functional iron deficiency.[31] The increased ferritin level in patients treated with rusfertide is also consistent with findings in patients with functional iron deficiency without evidence of iron overload. This conclusion reflects our observations that even after approximately 2 years of rusfertide treatment, the median serum ferritin concentration remained within normal limits. Furthermore, levels of transferrin saturation and serum iron were persistently low, findings that are inconsistent with iron overload.

Frequent phlebotomy because of poor hematocrit control can be a major burden on patients, prolonging physician visits and worsening iron deficiency, and can lead to additional nonhematologic symptoms.[25,32] Some patients are fearful of phlebotomy or unable to undergo the procedure. Phlebotomy often remains necessary even in patients receiving cytoreductive therapy, owing to the suboptimal control of hematocrit and symptoms.[16,17] Our data suggest that rusfertide therapy may improve hematocrit control and decrease the use of phlebotomy.

Available therapies have not been shown to definitively change the natural history of polycythemia vera. Whether the benefits of approved therapeutic agents result from consistent hematocrit control or from an effect on platelet or leukocyte counts remains unclear. Although other factors may potentially be important in decreasing the risk of thromboembolic events, evidence supporting the importance of hematocrit control (<45%), with clear guidelines for treatment, is more definitive. In seminal clinical trials of therapeutic agents in patients with polycythemia vera, efficacy was assessed on the basis of hematocrit control.[14,15] In the MAJIC-PV trial, ruxolitinib therapy had a potentially beneficial effect on event-free survival in patients in whom a complete hematologic response occurred within 12 months after the initiation of treatment.[33] A complete response occurred in only 43% of the patients who received ruxolitinib treatment, and the time until the first thrombotic event during the trial was significantly correlated only with the mean number of phlebotomies.

Limitations of our trial include the small number of patients and the short duration of follow-up, which preclude the assessment of the effects of rusfertide treatment on complications of polycythemia vera, such as thrombotic events and disease progression. Open-label treatment with rusfertide may have biased the patients' assessment of symptoms. Treatments for polycythemia vera are known to increase the risk of skin cancer.[34,35] Frequent dermatologic examinations were implemented in this trial because current clinical data are too limited to determine

whether rusfertide treatment also increases the risk of skin cancer.

Rusfertide appears to represent a step forward in the treatment of polycythemia vera, with a novel mechanism of action that could become an additional therapeutic tool for the control of this disease. The phase 3 VERIFY trial of rusfertide (ClinicalTrials.gov number, NCT05210790) is ongoing. Rusfertide is a potentially effective treatment option for achieving and sustaining hematocrit control in patients with polycythemia vera, reducing the use of phlebotomy and the occurrence of debilitating disease-related symptoms.

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APPENDIX

The authors' full names and academic degrees are as follows: Marina Kremyanskaya, M.D., Ph.D., Andrew T. Kuykendall, M.D., Naveen Pemmaraju, M.D., Ellen K. Ritchie, M.D., Jason Gotlib, M.D., Aaron Gerds, M.D., Jeanne Palmer, M.D., Kristen Pettit, M.D., Uttam K. Nath, M.D., Abdulraheem Yacoub, M.D., Arturo Molina, M.D., Samuel R. Saks, M.D., Nishit B. Modi, Ph.D., Frank H. Valone, M.D., Sarita Khanna, Ph.D., Suneel Gupta, Ph.D., Srdan Verstovsek, M.D., Ph.D., Yelena Z. Ginzburg, M.D., and Ronald Hoffman, M.D.

The authors' affiliations are as follows: the Division of Hematology and Medical Oncology, Tisch Cancer Institute, Icahn School of Medicine at Mount Sinai (M.K., Y.Z.G., R.H.), and the Division of Hematology and Medical Oncology, Weill Cornell Medical College (E.K.R.) — both in New York; the Department of Malignant Hematology, H. Lee Moffitt Cancer Center, Tampa, FL (A.T.K.); the Department of Leukemia, University of Texas M.D. Anderson Cancer Center, Houston (N.P., S.V.); the Division of Hematology, Stanford Cancer Institute, Stanford University School of Medicine, Stanford (J.G.), and Clinical Research and Development, Protagonist Therapeutics, Newark (A.M., S.R.S, N.B.M., F.H.V., S.K., S.G.) — both in California; the Department of Hematology and Medical Oncology, Cleveland Clinic Taussig Cancer Institute, Cleveland (A.G.); the Mayo Clinic, Phoenix, AZ (J.P.); the Rogel Cancer Center, University of Michigan Health, Ann Arbor (K.P.); All India Institute of Medical Sciences, Rishikesh, India (U.K.N.); and the University of Kansas Cancer Center, Westwood (A.Y.).

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