Online Supplement

COMFORT-I Investigators

The following investigators contributed to the study (listed in alphabetical order by country): **Australia**—P. Cannell, Royal Perth Hospital, Perth, WA; J. V. Catalano, Frankston Hospital and Department of Clinical Haematology, Monash University, Frankston, Victoria; B. H. Chong, St. George Hospital, Kogarah, NSW; P. Coughlin, Monash University/Box Hill Hospital, Box Hill, Victoria; S. T. S. Durrant, Royal Brisbane and Women's Hospital, Herston, Queensland; T. E. Gan, Monash Medical Centre, Clayton, Victoria; H. C. Lai, Townsville Hospital, Douglas, Queensland; M. F. Leahy, Fremantle Hospital and Health Service, Fremantle, WA; M. Leyden, Maroondah Hospital, Ringwood East, Victoria; R. Lindeman, Prince of Wales Hospital, Randwick, NSW; D. Ma, St. Vincent's Hospital, Darlinghurst, NSW; A. Perkins, Haematology and Oncology Clinics of Australia, Milton, Queensland; A. C. Perkins, Princess Alexandra Hospital, Woolloongabba, Queensland; D. Ross, Flinders Medical Centre, Bedford Park, SA; W. Stevenson, Royal North Shore Hospital, St. Leonards, NSW. **Canada**—K. Grewal, Eastern Health, St. John's, NL; V. Gupta, Princess Margaret Hospital, University of Toronto, Toronto, ON; K. Howson-Jan, London Health Sciences Centre, London, ON; S. Jackson, St. Paul's Hospital, Vancouver, BC; C. Shustik, Royal Victoria Hospital, Montreal, QC; R. van der Jagt, Ottawa Hospital-General Campus, Ottawa, ON. **United States**—L. Afrin, Hollings Cancer Center, Charleston, SC; L. P. Akard, Indiana Blood and Marrow Transplantation, LLC, Beech Grove, IN; M. O. Arcasoy, Duke University Medical Center, Durham, NC; E. Atallah, Froedtert Hospital and Medical College of Wisconsin, Milwaukee, WI; J. Altman, Northwestern Memorial Hospital, Chicago, IL; J. Camoriano, Mayo Clinic Arizona, Scottsdale, AZ; T. P. Cescon, Berks Hematology Oncology Associates, West Reading, PA; C. R. Cogle, University of Florida, Gainesville, FL; R. Collins, Jr., University of Texas Southwestern Medical Center, Dallas, TX; K-

H. Dao, Oregon Health and Science University, Portland, OR; H. J. Deeg, Fred Hutchinson Cancer Research Center, Seattle, WA; M. Deininger, Oregon Health and Science University, Portland, OR; N. J. DiBella, Rocky Mountain Cancer Centers, Aurora, CO; J. F. DiPersio, Washington University School of Medicine, St. Louis, MO; A. Faitlowicz, University of California- Irvine Medical Center, Orange, CA; F. A. Fakih, Florida Pulmonary Research Institute, LLC, Winter Park, FL; R. Frank, Norwalk Hospital, Norwalk, CT; N. Y. Gabrail, Gabrail Cancer Center Research, Canton, OH; S. L. Goldberg, Hackensack University Medical Center, Hackensack, NJ; J. Gotlib, Stanford Cancer Institute, Stanford, CA; H. M. Gross, Dayton Physicians, LLC, Dayton, OH; J. H. Harvey, Jr., Birmingham Hematology and Oncology Associates, LLC, Birmingham AL; R. H. Herzig, University of Louisville, Louisville, KY; E. Hexner, Abramson Cancer Center at the University of Pennsylvania, Philadelphia, PA; C. E. Holmes, Vermont Cancer Center, Burlington, VT; E. Ibrahim, Beaver Medical Group, Highland, CA; R. Jacobson, Palm Beach Cancer Institute, West Palm Beach, FL; C. Jamieson, Moores University of California-San Diego Cancer Center, La Jolla, CA; K. Jamieson, University of Iowa Hospitals and Clinic, Iowa City, IA; C. M. Jones, Jones Clinic, PC, Germantown, TN; H. M. Kantarjian, University of Texas M.D. Anderson Cancer Center, Houston, TX; A. Kassim, Vanderbilt Clinic, Nashville, TN; C. M. Kessler, Georgetown University Medical Center, Washington, DC; T. Kindwall-Keller, University Hospitals Case Medical Center, Cleveland, OH; P. P. N. Lee, Tower Cancer Research Foundation, Beverly Hills, CA; R. M. Lyons, Cancer Care Centers of South Texas/US Oncology, San Antonio, TX; R. Marschke, Jr., Front Range Cancer Specialists, Fort Collins, CO; J. Mascarenhas, Mount Sinai School of Medicine, New York, NY; E. Meiri, Palm Beach Institute of Hematology and Oncology, Boynton Beach, FL; A. Menter, Kaiser Permanente, Denver, CO; R. A. Mesa, Mayo

Clinic-Arizona, Scottsdale, AZ; C. Miller, St. Agnes HealthCare, Inc., Baltimore, MD; C. O'Connell, University of Southern California, Los Angeles, CA; I. Okazaki, Straub Clinic and Hospital, Honolulu, HI; R. Orlowski, Carolina Oncology Specialists, PA, Hickory, NC; R. Paquette, University of California-Los Angeles Medical Hematology and Oncology, Los Angeles, CA; V. R. Phooshkooru, Mid Dakota Clinic, PC, Bismarck, ND; B. Powell, Wake Forest University Health Services, Winston-Salem, NC; J. T. Prchal, Huntsman Cancer Institute, Salt Lake City, UT; R. Ramchandren, Karmanos Cancer Institute, Detroit, MI; F. Rana, Shands Jacksonville Clinical Center, Jacksonville, FL; A. Raza, Columbia University Medical Center, New York, NY; C. Rivera, Mayo Clinic-Jacksonville, Jacksonville, FL; E. A. Sahovic, Western Pennsylvania Hospital, Pittsburgh, PA; M. Scola, Carol G. Simon Cancer Center, Morristown, NJ; M. Scouros, Houston Cancer Institute, PA, Houston, TX; M. Sekeres, Cleveland Clinic, Cleveland, OH; J. Shammo, Rush University Medical Center, Chicago, IL; R. S. Siegel, George Washington University, Washington, DC; R. T. Silver, Weill Cornell Medical Center, New York, NY; C. P. Spears, Sierra Hematology and Oncology, Sacramento, CA; M. Talpaz, University of Michigan Medical Center, Ann Arbor, MI; M. Tsai, Park Nicollet Institute, St. Louis Park, MN; S. Verstovsek, University of Texas M.D. Anderson Cancer Center, Houston, TX; T. Walters, Mountain States Tumor Institute, Boise, ID; R. S. Weiner, Arena Oncology Associates, PC, Lake Success, NY; E. F. Winton, Emory University Hospital, Atlanta, GA; S. E. Young, Somerset Hematology-Oncology Associates, Somerville, NJ; F. Yunus, University of Tennessee Cancer Institute, Memphis, TN.

Detailed Methods

Patients

Inclusion and exclusion criteria have been described elsewhere.(1) Briefly, eligible patients were 18 years of age or older with PMF, post PV-MF or post ET-MF according to the 2008 World Health Organization criteria(2) and intermediate-2 or high-risk MF by International Prognostic Scoring System.(3) Patients also had to have a palpable spleen length ≥5 cm, platelet count ≥100×10^9/L and were refractory to or not candidates for available therapy.(1)

The protocol was approved by the institutional review board at each participating site. The study was conducted in accordance with the International Conference on Harmonisation guidelines for Good Clinical Practice. All patients provided written informed consent. Data were collected by the investigators and analyzed by the sponsor, Incyte Corporation. All authors had access to the data.

Study design

Patients were randomized 1:1 to receive ruxolitinib or placebo orally twice daily. Ruxolitinib starting doses were determined according to baseline platelet count: for patients with baseline platelets 100-200×10^9/L, the starting dose of ruxolitinib was 15 mg twice daily; for patients with baseline platelets >200×10^9/L, the starting dose of ruxolitinib was 20 mg twice daily. Doses were individualized to ensure safety and enhance efficacy. Doses could be increased for inadequate efficacy in patients with adequate platelet and absolute neutrophil counts. Dose holds were required for platelet counts <50×10^9/L or absolute neutrophil count <0.5×10^9/L, and dose adjustments were required for platelet counts <125×10^9/L (depending on the dose at the time of platelet count decline). Dose holds or adjustments were not required for anemia, although dose

adjustments and red blood cell (RBC) transfusions were permitted. Patients receiving placebo were eligible for crossover to ruxolitinib before week 24 if they had a ≥25% increase from baseline in spleen volume accompanied by worsening early satiety with weight loss or worsening spleen-related pain requiring narcotic analgesics; after week 24, an asymptomatic increase in spleen volume ≥25% alone was sufficient for crossover. All patients were eligible for crossover following completion of the primary analysis, when all patients had completed 24 weeks and at least half had completed 36 weeks of randomized treatment, at which time the study was unblinded.(1)

Evaluations

Spleen volume was measured by MRI or CT (for patients in whom MRI was contraindicated or not available). Imaging for spleen volume assessment was obtained at baseline and weeks 12, 24, 36, 48, 60 and 72, and every 24 weeks thereafter. MF symptom burden was measured daily up to week 24 with the modified MF Symptom Assessment Form version 2.0 electronic diary. The following symptoms were assessed on a scale of 0 (absent) to 10 (worst imaginable): night sweats, itching (pruritus), abdominal discomfort, pain under the ribs on the left side, feeling of fullness (early satiety), muscle/bone pain and inactivity. The sum of the individual symptom scores, excluding the score for inactivity, was used to determine the total symptom score (TSS). Patient QoL was evaluated with the self-administered European Organization for Research and Treatment of Cancer Quality of Life Questionnaire Core 30 (EORTC QLQ-C30) at baseline and each study visit. Adverse events were reported using National Cancer Institute Common Terminology Criteria for Adverse Events version 3.0.(1)

Statistical analysis

The data cutoff for this analysis of the ongoing COMFORT-I study was March 1, 2012 (1 year after a prospectively defined safety follow-up). Kaplan-Meier analysis was used to evaluate the durability of the spleen response and to assess OS. The analysis of durability of spleen volume reduction included all patients who had at least one spleen volume assessment demonstrating a ≥35% reduction from baseline. Duration of spleen volume response was defined as the time from first reduction of at least 35% from baseline to time of <35% reduction from baseline that was also a 25% increase over nadir. OS was determined according to original randomized treatment regardless of treatment crossover for all patients in the intent-to treat population and was censored at last known date alive. The Cox proportional hazards model was used to calculate HR and 95% CI and log-rank test for *P* value (unadjusted for repeat analyses).

Percentage changes in spleen volume from baseline to week 24 and 48 and percentage change in TSS from baseline to week 24 were evaluated by titrated dose. Titrated dose was defined as the average dose in the last 12 weeks prior to the assessment: <10 mg twice daily (average total daily dose ≤15 mg), 10 mg twice daily (>15-25 mg), 15 mg twice daily (>25-35 mg), 20 mg twice daily (>35-45 mg) and >20 mg twice daily (>45 mg).

Percentage changes from baseline in hemoglobin and platelet count as well as the proportion of patients who received any units of RBC transfusions during the previous 4 weeks were also assessed. In patients randomized to receive ruxolitinib, percentage changes from baseline in hemoglobin levels were also evaluated, including only patients who did not receive post-baseline RBC transfusions before week 36. The incidence of worsening grade 3 and grade 4 anemia and thrombocytopenia, as defined by laboratory values, was assessed at 6-month intervals (0-<6, 6-<12, 12-<18, 18-<24 and ≥24 months). Because all patients receiving placebo

had either crossed over to ruxolitinib treatment or discontinued from the study after the primary analysis and therefore only a subset of these patients had data beyond 6 months, the incidence of anemia and thrombocytopenia after 6 months was summarized only for patients originally randomized to receive ruxolitinib. Incidence was calculated using the life table method based on the time to first worsening grade 3 or 4 event censored at the time of discontinuation or data cutoff (earlier of the two); the effective sample size was used as the denominator. The incidence of overall and grade ≥3 nonhematologic events and treatment discontinuation rates by exposure interval were calculated in a similar manner. Median exposure time was calculated based on time to discontinuation using reverse Kaplan-Meier method.

References

1. Verstovsek S, Mesa RA, Gotlib J, Levy RS, Gupta V, DiPersio JF, et al. A double-blind, placebo-controlled trial of ruxolitinib for myelofibrosis. N Engl J Med. 2012 Mar 1;366(9):799-807.
2. Tefferi A, Vardiman JW. Classification and diagnosis of myeloproliferative neoplasms: the 2008 World Health Organizaiton criteria and point-of-care diagnostic algorithms. Leukemia. 2008 Jan;22(1);14-22.
3. Cervantes F, Dupriez B, Pereira A, Passamonti F, Reilly JT, Morra E, et al. New prognostic scoring system for primary myelofibrosis based on a study of the International Working Group for Myelofibrosis Research and Treatment. Blood. 2009 Mar 26;113(13);2895-901.

Supplementary Table 1. Causes of death by randomized treatment allocation.*

Cause of Death	**Ruxolitinib (N=155)**	**Placebo (N=154)**
Acute myeloid leukemia	2	3
Anastomotic hemorrhage		1
Cerebral hemorrhage		1
Completed suicide		1
Congestive heart failure resulting from pneumonia		1
Death	1	
Disease progression	4	7
Graft versus host disease	1	
Gastrointestinal hemorrhage		2
Leukemia or underlying leukemia	1	1
Intestinal perforation		1
Intra-abdominal hemorrhage		1
Muscular weakness	1	
MDS disease progression		1
Metastatic colon cancer		1
Multi-organ failure		1
Myelofibrosis	1	3
Myelofibrosis with possible transformation to acute myelogenous leukemia and pneumonia		1
Myeloproliferative disease		1

Non-small cell lung cancer metastatic	1	
Pneumonia	1	1
Pneumonia; septic shock	1	
Pneumonia, multi organ failure	1	
Renal failure	1	
Respiratory failure	1	
Road traffic accident		1
Shock hemorrhagic		1
Shock, respiratory and cardiac failure; hemorrhage following splenectomy	1	
Sepsis or septic shock	3	3
Splenic infarction	1	
Staphylococcal infection		1
Subdural hematoma	1	1
Surgical complications		1
Unknown	4	5
Total	**27**	**41**

*Documentation of cause of death was not available for all patients.

Supplementary Table 2. Incidence of new-onset grade 3/4 nonhematologic adverse events regardless of causality.

Patients (%)	0 to less than 6 months		6 to less than 12 months	12 to less than 18 months	18 to less than 24 months	24 months or more
	RUX	PBO	RUX	RUX	RUX	RUX
Fatigue	6.1	6.4	0	0.9	0	0
Pneumonia	4.1	3.6	1.6	3.6	1.3	0
Abdominal pain	2.7	9.9	1.6	0	1.2	3.6
Arthralgia	2.0	0	0	0	0	0
Diarrhea	2.0	0	0	0	0	0
Dyspnea	1.4	2.9	0.8	0	2.5	0
Fall	1.4	1.4	0	0.9	0	0
GI hemorrhage	1.4	0.7	0.8	0	0	0
Hyperuricemia	1.4	2.2	0	0	0	0
Muscular weakness	1.4	0	0	0	0	0
Septic shock	1.4	0	0	0	0	0
Hypotension	0.7	0.7	0	0	2.4	0
Hypoxia	0.7	0.7	0.8	0	2.5	0
Pain in extremity	0.7	0	1.5	0	0	0
Acute renal failure	0.7	2.2	0	0	2.5	3.6
Sepsis	0.7	0.7	0	0.9	2.5	0

Hyperglycemia	0	0	0	0	2.4	0

GI: gastrointestinal; *PBO:* placebo; *RUX:* ruxolitinib.

For each time interval, the effective sample size of the interval was used as the denominator. The effective sample size = the number of patients at risk at the beginning of the interval, plus half of the censored patients during the time interval.

Supplementary Table 3. Adverse events (grade 3/4 and serious) reported during treatment interruption.

Adverse event	Ruxolitinib (N=89)		Placebo (N=62)	
	Grade 3/4	**Serious**	**Grade 3/4**	**Serious**
Total patients with AEs, n (%)	8 (9.0)	3 (3.4)	7 (11.3)	2 (3.2)
Anemia	5 (5.6)	1 (1.1)	0	0
Abdominal pain	1 (1.1)	0	0	0
Delirium	1 (1.1)	0	0	0
Disseminated intravascular coagulation	1 (1.1)	0	0	0
Fatigue	1 (1.1)	0	0	0
GI hemorrhage	1 (1.1)	1 (1.1)	0	0
Renal failure acute	1 (1.1)	0	1 (1.6)	0
Thrombocytopenia	1 (1.1)	0	1 (1.6)	0
Nausea	1 (1.1)	0	0	0
Urosepsis	0	1 (1.1)	0	0
Asthenia	0	0	1 (1.6)	0
Atrial fibrillation	0	0	1 (1.6)	0
Gastric varices	0	0	1 (1.6)	0
Gout	0	0	1 (1.6)	1 (1.6)
Hepatic encephalopathy	0	0	1 (1.6)	1 (1.6)
Hyperbilirubinemia	0	0	1 (1.6)	0

Splenic infarction	0	0	1 (1.6)	0
Ventricular dysfunction	0	0	1 (1.6)	0
Vomiting	1 (1.1)	1 (1.1)	1 (1.6)	1 (1.6)
Ascites	0	0	1 (1.6)	0
Hydronephrosis	0	0	1 (1.6)	0
Febrile neutropenia	0	1 (1.1)	0	0
Pulmonary edema	0	0	0	1 (1.6)

AE: adverse event; *GI:* gastrointestinal.

Numbers reported are percentages of those who had a treatment interruption (not the total study population).

Supplementary Table 4. Adverse events (grade 3/4 and serious) reported after study discontinuation*.

Adverse event	Ruxolitinib (N=55)		Placebo (N=40)	
	Grade 3/4	**Serious**	**Grade 3/4**	**Serious**
Total patients with AEs, n (%)	20 (36.4)	20 (36.4)	20 (50)	15 (30)
Thrombocytopenia	4 (7.3)	2 (3.6)	2 (5.0)	0
Acute myeloid leukemia	2 (3.6)	2 (3.6)	0	0
Dyspnea	2 (3.6)	1 (1.8)	2 (5.0)	0
Pneumonia	2 (3.6)	3 (5.5)	4 (10.0)	2 (5.0)
Splenic infarction	2 (3.6)	2 (3.6)	0	0
Abdominal pain	1 (1.8)	0	4 (10.0)	2 (5.0)
Cardiac arrest	1 (1.8)	0	0	0
Clostridial infection	1 (1.8)	1 (1.8)	0	0
Death	1 (1.8)	1 (1.8)	0	0
Disease progression	1 (1.8)	1 (1.8)	2 (5.0)	2 (5.0)
Disseminated intravascular coagulation	1 (1.8)	0	0	0
Edema	1 (1.8)	0	0	0
Epistaxis	1 (1.8)	0	0	0
Fatigue	1 (1.8)	1 (1.8)	3 (7.5)	0
Hemoglobin decreased	1 (1.8)	0	0	0
Hepatosplenomegaly	1 (1.8)	1 (1.8)	0	0

Hyperglycemia	1 (1.8)	0	0	0
Hypokalemia	1 (1.8)	0	0	0
Hypotension	1 (1.8)	0	0	0
Hypoxia	1 (1.8)	0	2 (5.0)	0
Lactic acidosis	1 (1.8)	0	0	0
Malnutrition	1 (1.8)	0	1 (2.5)	0
Muscular weakness	1 (1.8)	1 (1.8)	0	0
Myocardial infarction	1 (1.8)	1 (1.8)	0	0
Platelet count increased	1 (1.8)	0	0	0
Portal vein thrombosis	1 (1.8)	0	0	0
Pulmonary edema	1 (1.8)	0	1 (2.5)	1 (2.5)
Pyrexia	1 (1.8)	2 (3.6)	0	0
Renal failure	1 (1.8)	1 (1.8)	2 (5.0)	2 (5.0)
Renal failure acute	1 (1.8)	0	0	0
Respiratory failure	1 (1.8)	1 (1.8)	0	0
Sepsis	1 (1.8)	1 (1.8)	1 (2.5)	1 (2.5)
Septic shock	1 (1.8)	1 (1.8)	0	0
Splenic hemorrhage	1 (1.8)	1 (1.8)	0	0
Subdural hematoma	1 (1.8)	1 (1.8)	2 (5.0)	2 (5.0)
Transaminases increased	1 (1.8)	0	0	0
Transient ischemic attack	1 (1.8)	1 (1.8)	0	0
Abdominal pain upper	0	1 (1.8)	0	0
Agitation	0	0	1 (2.5)	0

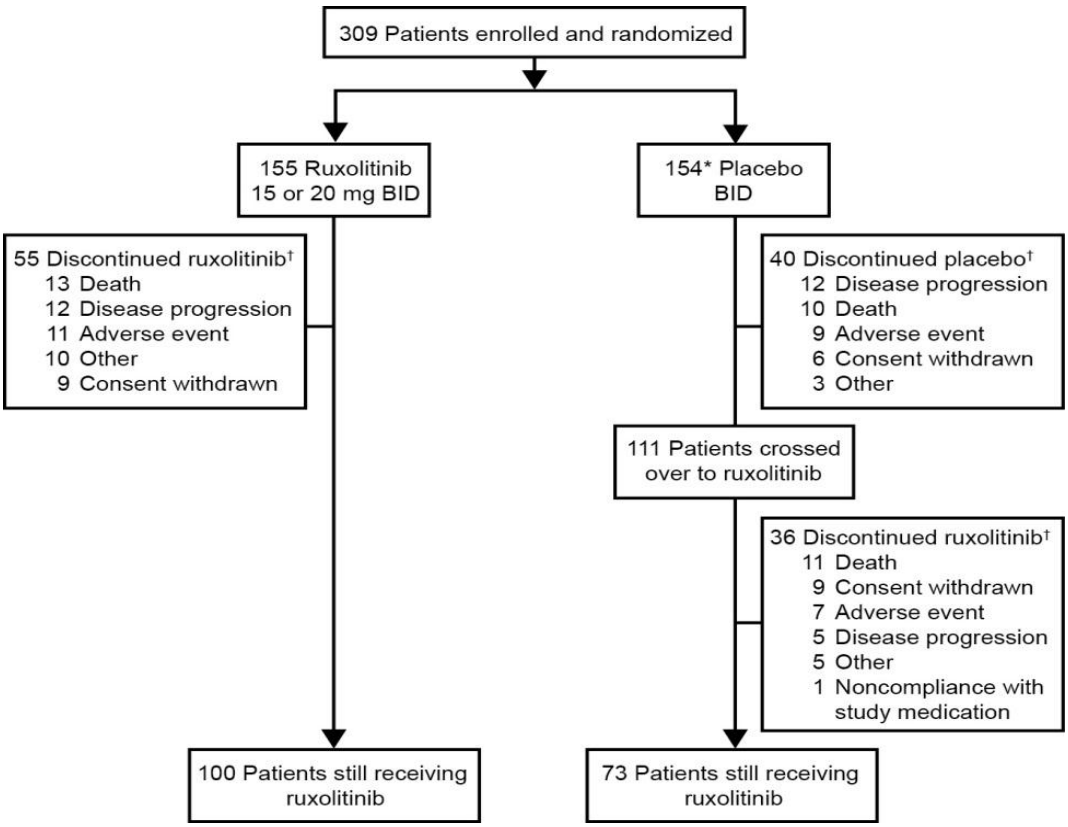
Anemia	0	1 (1.8)	0	0
Arthralgia	0	0	1 (2.5)	0
Atrial fibrillation	0	0	1 (2.5)	1 (2.5)
Blood amylase increased	0	0	1 (2.5)	0
Blood magnesium decreased	0	0	1 (2.5)	0
Cardiac failure	0	0	1 (2.5)	1 (2.5)
Cellulitis	0	1 (1.8)	0	0
Chronic obstructive pulmonary disease	0	0	1 (2.5)	0
Colitis	0	0	1 (2.5)	1 (2.5)
Dehydration	0	1 (1.8)	2 (5.0)	1 (2.5)
Diarrhea	0	1 (1.8)	0	0
Fall	0	1 (1.8)	2 (5.0)	1 (2.5)
Febrile neutropenia	0	0	1 (2.5)	0
GI hemorrhage	0	0	1 (2.5)	1 (2.5)
Hyponatremia	0	0	2 (5.0)	0
Intestinal ischemia	0	0	1 (2.5)	1 (2.5)
Leukocytosis	0	0	1 (2.5)	1 (2.5)
Lipase increased	0	0	1 (2.5)	0
Loss of consciousness	0	0	1 (2.5)	0
Multi-organ failure	0	0	1 (2.5)	1 (2.5)
Musculoskeletal pain	0	0	1 (2.5)	0
Myelofibrosis	0	0	1 (2.5)	1 (2.5)

Postoperative wound infection	0	1 (1.8)	0	0
Pulmonary embolism	0	0	2 (5.0)	1 (2.5)
Splenic hematoma	0	0	1 (2.5)	1 (2.5)
Splenomegaly	0	0	1 (2.5)	0
Staphylococcal infection	0	0	1 (2.5)	1 (2.5)
Tachycardia	0	0	1 (2.5)	0
Urinary tract infection	0	0	1 (2.5)	1 (2.5)
Weight increased	0	0	1 (2.5)	0

AE: adverse event; *GI:* gastrointestinal.

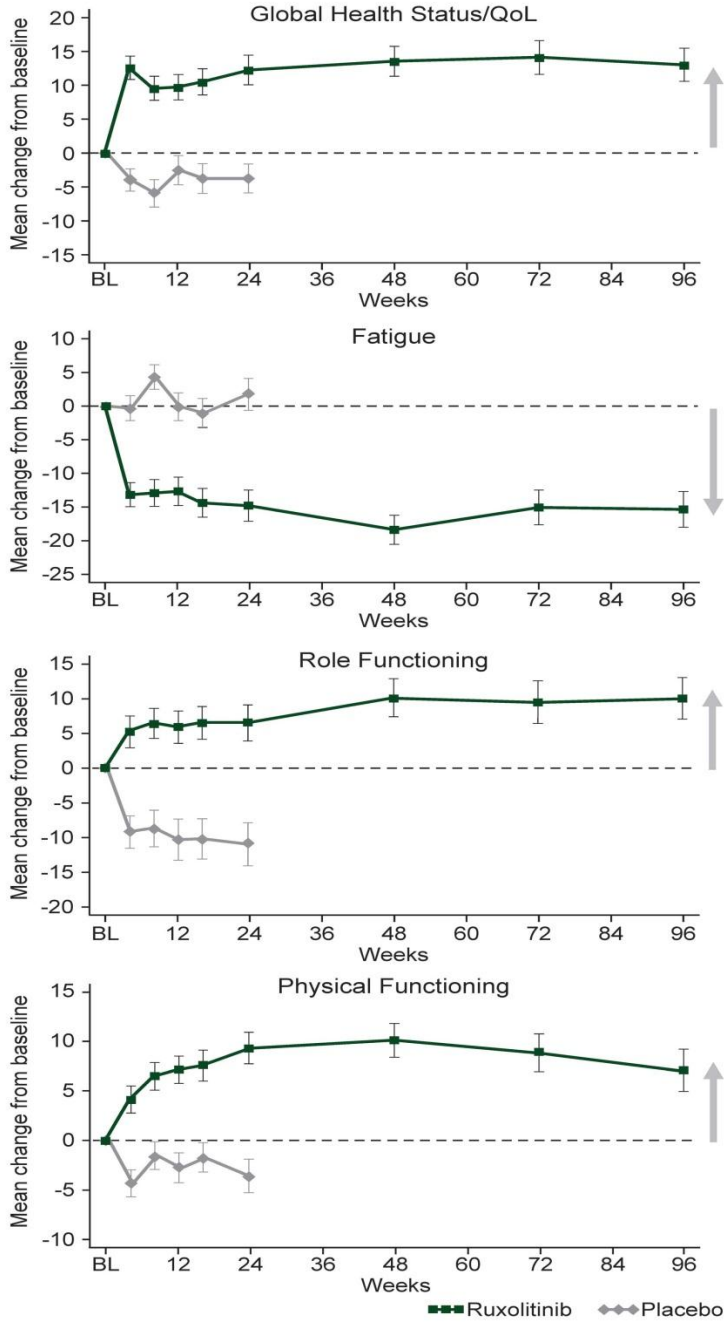
*Numbers reported are percentages of those who discontinued the study (not the total study population).

Supplementary Figure 1. Patient disposition.

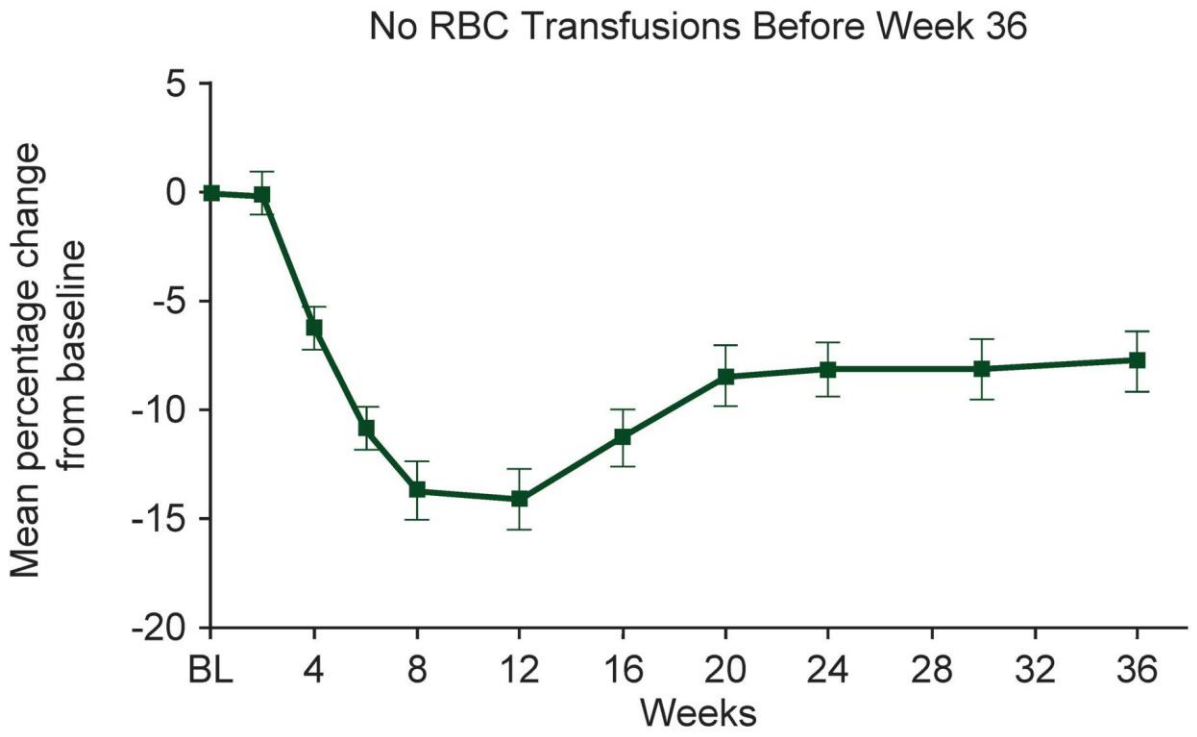


*Three patients were not evaluable for safety but were included in the intent-to-treat analysis of efficacy. †Discontinuations represent absolute numbers unadjusted for differences in exposure. “Other” reasons for discontinuation in the ruxolitinib group: decision to receive transplant (3), refractory to medication (2), patient choice to pursue different treatment, patient entered hospice, investigator decision, worsening symptoms, lack of efficacy; in the placebo group: patient choice (2), patient put on hydroxyurea; and in the crossover group: patient entered hospice, no improvement in blood counts, patient choice, refractory to medication, investigator decision. *BID*: twice daily.

Supplementary Figure 2. Mean changes (±SEM) in EORTC QLQ-C30 scores over time. (A) Global health status/QoL, (B) fatigue symptom score, (C) role functioning, and (D) physical functioning. Arrows indicate direction of improvement. *QoL:* quality of life.



Supplementary Figure 3. Mean percentage change (±SEM) from baseline in hemoglobin levels over time in patients randomized to receive ruxolitinib who completed first 36 weeks of treatment and did not received post-baseline RBC transfusions before week 36. *RBC:* red blood cell.



Supplementary Figure 4. **The proportion of patients receiving RBC transfusions in the prior month by randomized group over time.** *RBC:* red blood cell.