Published Ahead of Print on September 20, 2013, as doi:10.3324/haematol.2013.095109.
Copyright 2013 Ferrata Storti Foundation.

haematologica
the hematology journal

Early Release Paper

Resolution of bone marrow fibrosis in a patient receiving JAK1/JAK2 inhibitor treatment with ruxolitinib

by Bridget S. Wilkins, Deepti Radia, Claire Woodley, Sarah El Farhi, Clodagh Keohane, and Claire N. Harrison

Haematologica 2013 [Epub ahead of print]

Citation: Wilkins BS, Radia D, Woodley C, El Farhi S, Keohane C, and Harrison CN. Resolution of bone marrow fibrosis in a patient receiving JAK1/JAK2 inhibitor treatment with ruxolitinib. Haematologica. 2013; 98:xxx
doi:10.3324/haematol.2013.095109

Publisher's Disclaimer.
E-publishing ahead of print is increasingly important for the rapid dissemination of science. Haematologica is, therefore, E-publishing PDF files of an early version of manuscripts that have completed a regular peer review and have been accepted for publication. E-publishing of this PDF file has been approved by the authors. After having E-published Ahead of Print, manuscripts will then undergo technical and English editing, typesetting, proof correction and be presented for the authors' final approval; the final version of the manuscript will then appear in print on a regular issue of the journal. All legal disclaimers that apply to the journal also pertain to this production process.

Haematologica (pISSN: 0390-6078, eISSN: 1592-8721, NLM ID: 0417435, www.haematologica.org) publishes peer-reviewed papers across all areas of experimental and clinical hematology. The journal is owned by the Ferrata Storti Foundation, a non-profit organization, and serves the scientific community with strict adherence to the principles of open access publishing (www.doaj.org). In addition, the journal makes every paper published immediately available in PubMed Central (PMC), the US National Institutes of Health (NIH) free digital archive of biomedical and life sciences journal literature.

Support Haematologica and Open Access Publishing by becoming a member of the European Hematology Association (EHA) and enjoying the benefits of this membership, which include free participation in the online CME program

Official Organ of the European Hematology Association
Published by the Ferrata Storti Foundation, Pavia, Italy
www.haematologica.org

Resolution of bone marrow fibrosis in a patient receiving JAK1/JAK2 inhibitor treatment with ruxolitinib

Bridget S. Wilkins,* Deepti Radia, Claire Woodley, Sarah El Farhi, Clodagh Keohane, and Claire N. Harrison

Guy's and St. Thomas' NHS Foundation Trust, London, UK

Correspondence

Bridget S. Wilkins, Cellular Pathology Department Second Floor, North Wing St Thomas' Hospital, London SE1 7EH. E-mail: bridget.wilkins@gstt.nhs.uk

Abstract

Ruxolitinib, a JAK1/JAK2 inhibitor, is currently the only pharmacological agent approved for the treatment of myelofibrosis. Approval was based on findings from two phase 3 trials comparing ruxolitinib with placebo (COMFORT-I) and with best available therapy (COMFORT-II) for the treatment of primary or secondary myelofibrosis. In those pivotal trials, ruxolitinib rapidly improved splenomegaly, disease-related symptoms, and quality of life and prolonged survival compared with both placebo and conventional treatments. However, for reasons that are currently unclear, there were only modest histomorphologic changes in the bone marrow, and only a subset of patients had significant reductions in *JAK2* V617F clonal burden. Here, we describe a patient with post-polycythemia vera myelofibrosis who received ruxolitinib at our institution (Guy's and St. Thomas' NHS Foundation Trust, London, United Kingdom) as part of the COMFORT-II study (NCT00934544). While on treatment, the patient had dramatic improvements in splenomegaly and symptoms shortly after starting ruxolitinib. With longer treatment, the patient had marked reductions in *JAK2* V617F allele burden, and fibrosis of the bone marrow resolved after approximately 3 years of ruxolitinib treatment. To our knowledge, this is the first detailed case report of resolution of fibrosis with a JAK1/JAK2 inhibitor.

Trial registration: ClinicalTrials.gov Identifier: NCT00934544

Introduction

Primary myelofibrosis (PMF) is a myeloproliferative neoplasm that is characterized clinically by bone marrow fibrosis, progressive anemia, and extramedullary hematopoiesis, which often manifests as splenomegaly.[1,2] Myelofibrosis (MF) may also develop as a manifestation of disease progression in other myeloproliferative neoplasms, particularly polycythemia vera (PV) and essential thrombocythemia (ET). At the stage of MF in all of these conditions, symptoms arise largely from fibrotic bone marrow replacement. Primary symptoms include those resulting from anemia or splenomegaly (eg, early satiety, abdominal pain) and constitutional symptoms (ie, fever, weight loss, night sweats).[3,4] Alleviation of these symptoms is a near-term goal of treatment, mainly because conventional therapies have been unable to meaningfully affect the biology of the disease.[2]

Allogeneic hematopoietic stem cell transplantation (AHSCT) is currently the only treatment option that has curative potential in MF.[5] However, AHSCT is associated with significant morbidities, and most patients are not suitable candidates. Additionally, with the exception of AHSCT, no therapy has consistently been associated with stabilization or resolution of fibrosis in patients with MF. Findings that reflect resolution of bone marrow fibrosis with Janus kinase (JAK) inhibitor treatment have not been reported. This report describes a patient with post-PV MF who had a marked reduction in bone marrow fibrosis during treatment with the JAK1/JAK2 inhibitor ruxolitinib. To our knowledge, this is the first detailed report of such a case.

Methods

The patient was enrolled in the COMFORT-II trial and was randomized to the ruxolitinib arm.[6] COMFORT-II is a randomized, open-label, multicenter study evaluating the safety and efficacy of ruxolitinib 15 or 20 mg twice daily (bid) compared with best available therapy for the treatment of PMF, post-PV MF, and post-ET MF. The primary endpoint was the proportion of patients who achieved a ≥ 35% reduction from baseline in spleen volume (ie, a spleen response) at week 48, as measured by magnetic

resonance imaging (MRI), and the key secondary endpoint was a spleen response at week 24. Secondary endpoints included overall survival, histopathologic characteristics of bone marrow, and analyses of symptoms and quality of life; dynamics of *JAK2* V617F clonal burden was an exploratory endpoint of the trial. The study design and patient criteria were fully described previously.[6] Of note, the starting dose was determined by the patient's platelet count at baseline (15 mg bid for patients with a platelet count from 100 to 200 × 10^9/L and 20 mg bid for patients with a platelet count > 200 × 10^9/L), and the dose was titrated for each patient throughout the trial to optimize safety and efficacy. Dose reductions were required for thrombocytopenia and followed a strict protocol-defined dosing regimen. The last data cutoff for the COMFORT-II study was 1 December 2012; here, we report the latest on-study results for this patient as well as additional findings from our institution.

The study protocol was approved by the institutional review board prior to patient enrollment and the study was conducted in accordance with the principles set forth by the Declaration of Helsinski.

Results—Case Report

A 74-year-old male patient presented to our clinic with constitutional symptoms (night sweats and fever), pruritus, and marked splenomegaly of 26 cm below the left costal margin (spleen volume: 3390 cm^3). He had received a diagnosis of PV 10 years previously, in 1999, and had been receiving treatment with hydroxycarbamide (HC) since the initial diagnosis. Comorbidities included hypertension and monoclonal gammopathy, both of which were found at the time of PV diagnosis.

A diagnosis of post-PV MF with a risk category of International Prognostic Scoring System (IPSS) criteria[3] intermediate-2 (age > 65 years and presence of constitutional symptoms) was confirmed, and the patient was enrolled in the COMFORT-II trial[6] at a starting dose of ruxolitinib 15 mg bid (platelet count at baseline: 138 × 10^9/L). The initial hemoglobin level was 140 g/L, and the white blood cell count was 15.6 × 10^9/L. At screening, the patient was found to be *JAK2* V617F–positive. Cytogenetic analysis showed an additional abnormality of 46,XY,der(22)t(1;22)(q21;p11.2) [4]/46,XY[3]; the abnormal clone was detected in 4 out of 7 cells analyzed by G-banding, with an unbalanced translocation between chromosomes 1 and 22 that resulted in partial trisomy 1q—a recognized finding in MF.[7]

After the initiation of ruxolitinib treatment, splenomegaly improved dramatically (Figure 1)—a 30% reduction in palpable spleen length was observed at week 4 (the first spleen assessment). However, the patient became mildly thrombocytopenic (Figure 2) with a platelet count of 86 × 10^9/L, and the dose was reduced to 10 mg bid as per study protocol. Platelet counts recovered with dose reduction, and the patient has remained on treatment at a dose of 10 mg bid. At this dose, splenomegaly progressively resolved. The smallest palpable spleen length measurement, 2 cm (a 90% reduction from baseline), was recorded after 108 weeks of ruxolitinib treatment. The patient was classified as having a spleen response, as defined per protocol, with a 42% reduction in spleen volume at week 24, a 58% reduction at week 48, and a 75% reduction at the latest assessment by MRI at week 144 (Figure 3). In terms of symptoms, both fatigue and intractable pruritus were present at study entry; within 48 hours of the initiation of ruxolitinib treatment, the pruritus had resolved (and has remained negligible) and the fatigue had improved markedly.

After 168 weeks of ruxolitinib treatment, morphologic analysis of the bone marrow indicated a significant improvement (Figure 4). At the pretrial baseline assessment in 2009, analysis of hematoxylin and eosin–stained trephine core sections showed maximally increased cellularity with disordered hematopoiesis and pleomorphic megakaryocytes (MKs), most of which were large and had abnormally lobated nuclei. Many formed tight clusters, and CD61 immunohistochemical analysis (highlighting MKs and platelets)

emphasized the increase, pleomorphism, and clustering. There was a diffuse and dense increase in reticulin with extensive intersections (Gomori reticulin stain) and focal formation of collagen (Martius Scarlet Blue trichrome stain), but no osteosclerosis (overall World Health Organization [WHO] score: 3).[8] After 48 weeks of ruxolitinib treatment, these abnormalities were still apparent, cellularity decreased, and reticulin fibers were less prominent; however, patches of dense reticulin remained with extensive intersections. After longer treatment durations (168 weeks), hematoxylin and eosin staining showed normal overall cellularity and more uniformly dispersed MKs (best seen with CD61 immunostaining). Occasional atypical MKs were still present, but clustering was minimal and most MKs had normal morphologic characteristics. CD61 immunostaining confirmed that MKs were more dispersed, were not present in tight clusters, and had limited pleomorphism; platelet shedding into the marrow interstitium was minimal compared with baseline. Silver staining showed complete normalization of the fiber pattern (WHO score: 0), and only normal perivascular collagen was evident with Martius Scarlet Blue staining. In parallel with this, the red cell changes characteristic of myelofibrosis (tear drop poilkilocytes and nucleated red cells) have also resolved, and the LDH normalized as documented in centrally reviewed laboratory findings.

Of interest, *JAK2* V617F allele burden was much reduced with ruxolitinib treatment: from an absolute allele burden of 91% at baseline to approximately 11% at week 156—an 88% reduction. This reduction occurred gradually over the course of treatment (Figure 1). The cytogenetic abnormality persisted despite the resolution of fibrosis.

Ruxolitinib treatment was generally well tolerated by this patient. Hematologic adverse events (AEs) included thrombocytopenia, which resolved after dose reduction and anemia. These AEs are expected in the context of JAK1/JAK2 inhibitor therapy, but experience from the COMFORT studies has shown that they are manageable in most patients, and the incidence decreases after 6 months of treatment.[9] There was a gradual decline in hemoglobin levels, from 140 g/L at baseline to 96 g/L at day 78. However, levels recovered shortly thereafter to ≥ 108 g/L for the remainder of treatment (Figure 2); the patient has not required any transfusions. Nonhematologic AEs included two lower respiratory tract infections (on study days 112 and 826) that resolved with antibiotic treatment. Other AEs of interest that were considered unrelated or unlikely to be related to treatment included basal cell carcinoma (resolved with Moh surgery) and squamous cell carcinoma (resolved with excision of the lesion on the right side of the chest).

Discussion

Dysregulation of the JAK/STAT pathway is a hallmark of MF,[10,11] and the resulting overexpression of several pro-inflammatory cytokines has been implicated in the progression of fibrosis.[12] Given the reported effects of ruxolitinib treatment on various pro-inflammatory cytokines,[6,13,14] one might expect an improvement in bone marrow fibrosis in some patients. At the time of the primary analyses of the COMFORT studies, no major changes in the morphologic characteristics of bone marrow were observed (after 6 months and 1 year of treatment in COMFORT-I and -II, respectively).[6,13] Reduced-intensity AHSCT has been shown to resolve fibrosis within 6 to 12 months in patients with MF[15]; a longer treatment duration may be necessary to observe significant improvements in fibrosis in the context of JAK1/JAK2 inhibitor therapy, where the pathway cannot be completely repressed. For example, in one retrospective analysis of patients at the MD Anderson Cancer Center receiving ruxolitinib in the phase I/II study, 15% of patients (10/67) had an improvement in fibrosis at 24 months and 24% (4/17) had an improvement at 48 months (assessed histologically on the basis of WHO fibrosis scoring). For comparison, 10% of patients (3/31) in a control group who received HC showed an improvement after 24 months, and no patients (0/20) showed an improvement at 48 months.[16] Of note, in that analysis, 53% of ruxolitinib-treated patients showed stabilization in morphologic characteristics of bone marrow at 48 months compared with

35% of HC-treated patients. Published literature on interferon therapy is also notable for occasional reports of resolution of histolopathological features in either PV (as documented in 3 patients by Larsen and colleagues[17]) or myelofibrosis; Silver and colleagues[18] reported morphological remission in 2 of 17 patients with early MF (WHO grade 1 or 2 fibrosis and IPSS low or intermediate 1 at diagnosis). A large French group[19] collated data regarding responses to interferon in myelofibrosis but did not document histological responses.

Here, we report for the first time the resolution of bone marrow fibrosis with normalization of hematopoiesis in a patient receiving ruxolitinib treatment in COMFORT-II, a pre-requisite for these studies was the presence of advanced disease (IPSS score intermediate 2 or above). The COMFORT studies have shown that ruxolitinib prolongs survival in patients with MF compared with placebo and best available therapy, which suggests a potential disease-modifying effect.[9,20] Longer-term follow up of the COMFORT study cohorts should help to determine whether ruxolitinib treatment can indeed improve the morphologic characteristics of bone marrow for other patients with MF and affect the natural course of the disease; if it does, it will be of great interest to define what the particular characteristics of such responding patients might be.

Authorship: BSW and CNH are responsible for the integrity of the work, as a whole. BSW, DR, CK and CNH interpreted the data and prepared the text. BSW and CNH collected the images and generated the figures. All authors collected the data and approved the manuscript.

Disclosures: Dr. Claire Harrison has received honoraria from Novartis, Sanofi-Aventis, Celgene and Shire; received research funding from Novartis and Shire; acted as a consultant to YM BioSciences, S*BIO, Sanofi-Aventis and Novartis**.** Dr. Clodagh Keohane has received research funding from Novartis. Dr. Radia has received honoraria from Novartis, Shire and Pharmacosmos.

References

1. Tefferi A. Essential thrombocythemia, polycythemia vera, and myelofibrosis: current management and the prospect of targeted therapy. Am J Hematol. 2008;83(6):491-7.

2. Vannucchi AM. Management of myelofibrosis. Hematol Am Soc Hematol Educ Program. 2011;2011:222-30.

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;113(13):2895-901.

4. Mesa RA, Schwager S, Radia D, Cheville A, Hussein K, Niblack J, et al. The Myelofibrosis Symptom Assessment Form (MFSAF): an evidence-based brief inventory to measure quality of life and symptomatic response to treatment in myelofibrosis. Leuk Res. 2009;33(9):1199-203.

5. McLornan DP, Mead AJ, Jackson G, Harrison CN. Allogeneic stem cell transplantation for myelofibrosis in 2012. Br J Haematol. 2012;157(4):413-25.

6. Harrison C, Kiladjian JJ, Al-Ali HK, Gisslinger H, Waltzman R, Stalbovskaya V, et al. JAK inhibition with ruxolitinib versus best available therapy for myelofibrosis. N Engl J Med. 2012;366(9):787-98.

7. Andrieux J, Demory JL, Caulier MT, Agape P, Wetterwald M, Bauters F, et al. Karyotypic abnormalities in myelofibrosis following polycythemia vera. Cancer Genet Cytogenet. 2003;140(2):118-23.

8. Thiele J, Kvasnicka HM, Facchetti F, Franco V, van der Walt J, Orazi A. European consensus on grading bone marrow fibrosis and assessment of cellularity. Haematologica. 2005;90(8):1128-32.

9. Verstovsek S, Mesa R, Gotlib J, Levy R, Gupta V, DiPersio J, et al. Long-term outcome of ruxolitinib treatment in patients with myelofibrosis: durable reductions in spleen volume, improvements in quality of life, and overall survival advantage in COMFORT-I, [abstract 800]. 54th ASH Annual Meeting and Exposition, Atlanta, GA; 2012 Dec 8-11.

10. Vannucchi AM. Management of myelofibrosis. Hematol Am Soc Hematol Educ Program. 2011;2011:222-30.

11. Vainchenker W, Delhommeau F, Constantinescu SN, Bernard OA. New mutations and pathogenesis of myeloproliferative neoplasms. Blood. 2011;188(7):1723-35.

12. Hasselbalch HC. The role of cytokines in the initiation and progression of myelofibrosis. Cytokine Growth Factor Rev. 2013;24(2):133-45.

13. Verstovsek S, Mesa RA, Gotlib J, Levy RS, Gupta V, DiPersio J, et al. A double-blind, placebo-controlled trial of ruxolitinib for myelofibrosis. N Engl J Med. 2012;366(9):799-807.

14. Verstovsek S, Kantarjian H, Mesa RA, Pardanani AD, Cortes-Franco J, Thomas DA, et al. Safety and efficacy of INCB018424, a JAK1 and JAK2 inhibitor, in myelofibrosis. N Engl J Med. 2010;363(12):1117-27.

15. Kroger N, Kvasnicka M, Thiele J. Replacement of hematopoietic system by allogeneic stem cell transplantation in myelofibrosis patients induces rapid regression of bone marrow fibrosis. Fibrogenesis Tissue Repair. 2012;5 Suppl 1:S25.

16. Kvasnicka HM, Thiele J, Bueso-Ramos C, Hou K, Cortes J, Kantarjian H, Verstovsek S. Exploratory analysis of the effect of ruxolitinib on bone marrow morphology in patients with myelofibrosis [abstract 703]. J Clin Oncol. 2013;31 Suppl.

17. Larsen TS, Iversen KF, Hansen E, et al. Long term molecular responses in a cohort of danish patients with essential thrombocythemia, polycythemia vera and myelofibrosis treated with recombinant interferon alpha. Leuk Res. 2013;37(9):1041-5.

18. Silver RT, Vandris K, Goldman JJ. Recombinant interferon-alpha may retard progression of early primary myelofibrosis: a preliminary report Blood. 2011;117(24):6669-72.

19. Ianotto JC, Boyer-Perrard F, Gyan E, et al. Efficacy and safety of pegylated-interferon alpha-2a in myelofibrosis: a study by the FIM and GEM French cooperative groups. Br J Haematol. 2013;162(6):783-91.

20. Cervantes F, Kiladjian J, Niederwieser D, Sirulnik A, Stalbovskaya V, McQuitty M, et al. Long-term efficacy, safety, and survival findings from COMFORT-II, a phase 3 study comparing ruxolitinib with best available therapy for the treatment of myelofibrosis [abstract 801]. 54th ASH Annual Meeting and Exposition, Atlanta, GA; 2012 Dec 8-11.

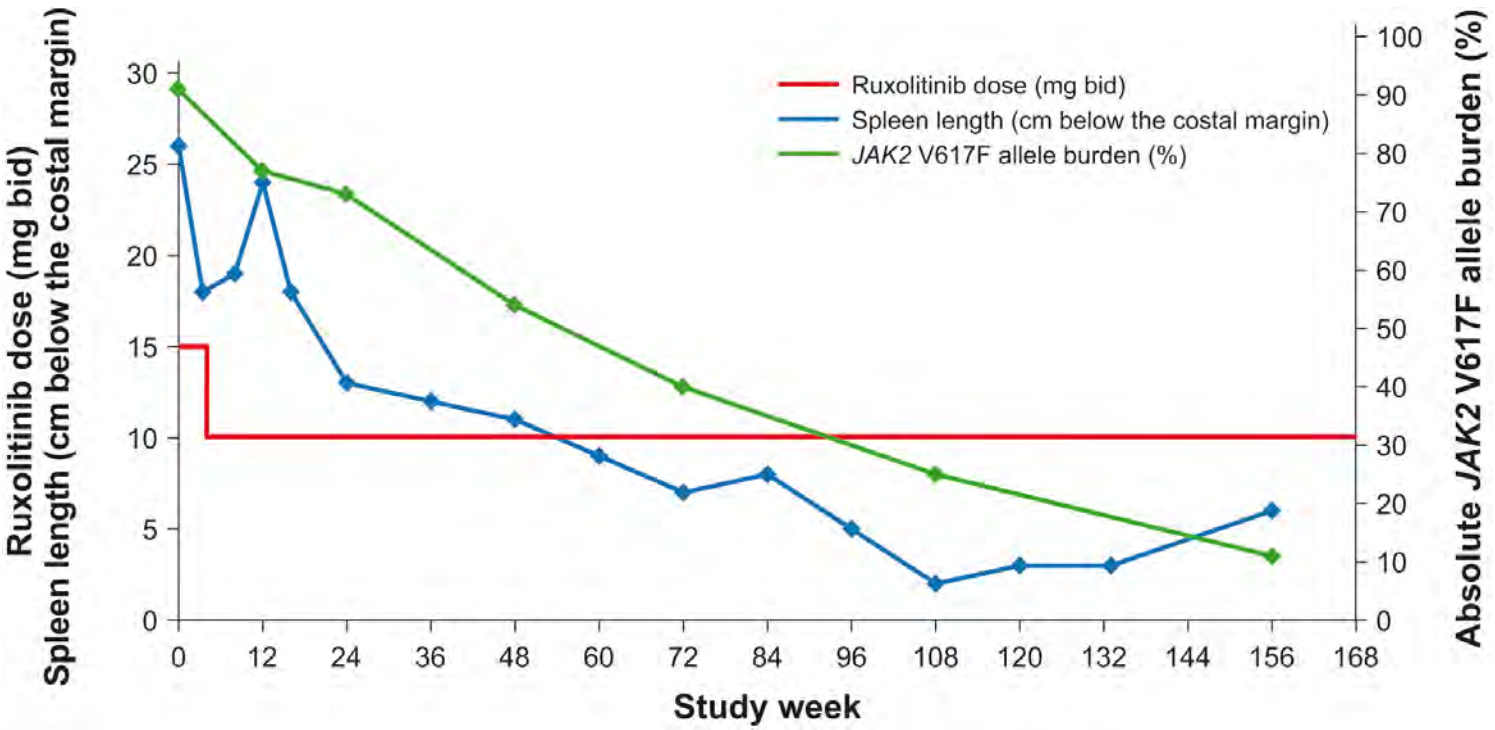
Figure Legends

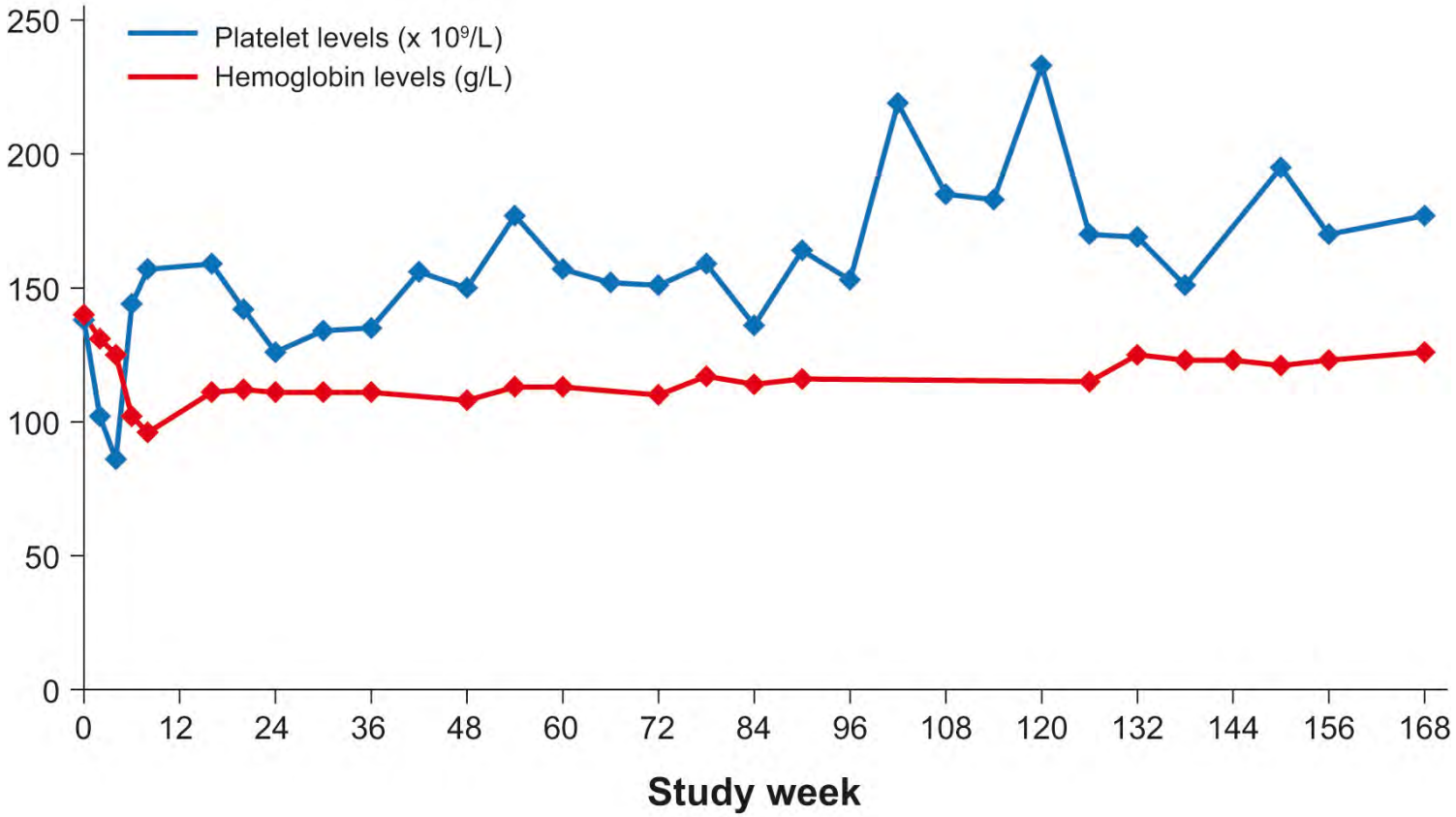
Figure 1. Ruxolitinib dose, palpable spleen length, and *JAK2* V617F allele burden over time. bid, twice daily.

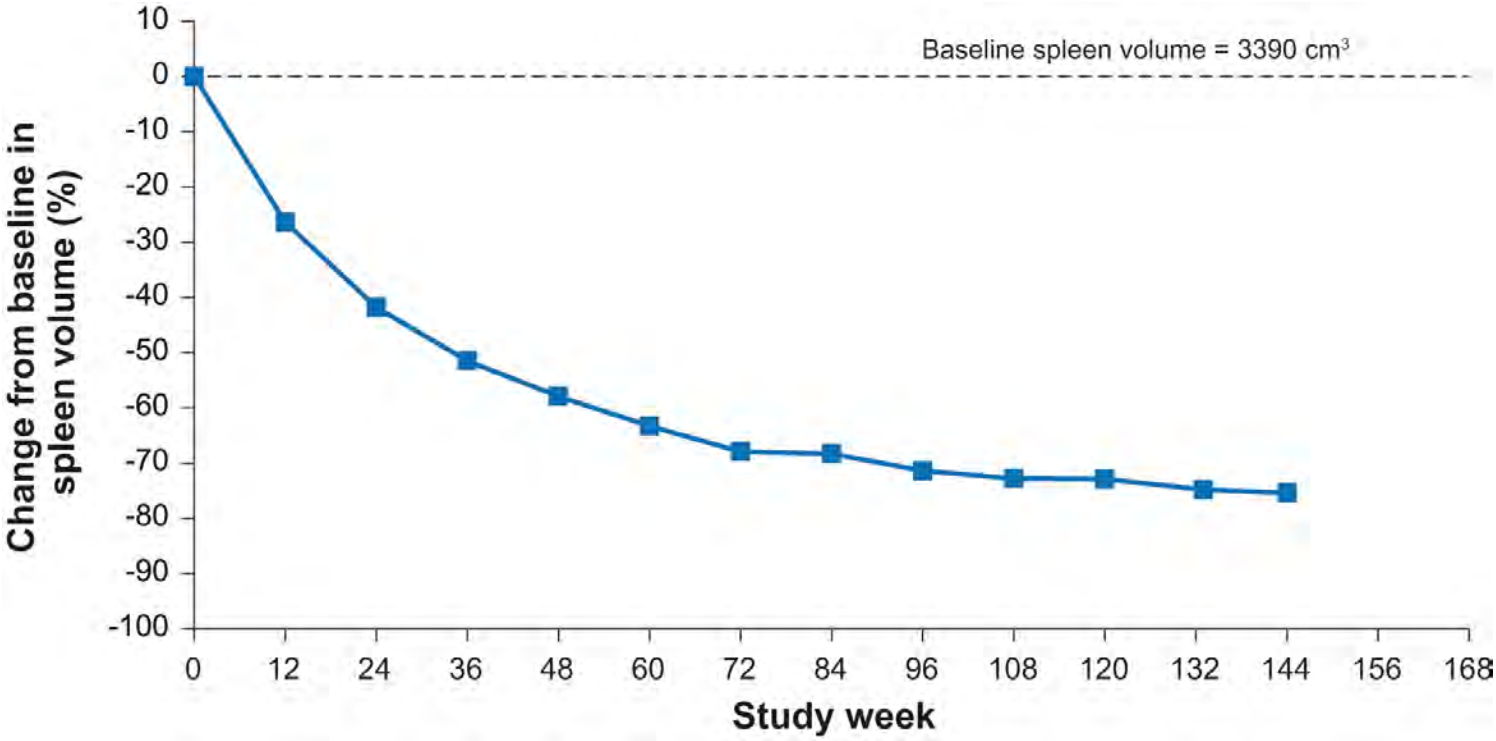
Figure 2. Platelet and hemoglobin levels over time.

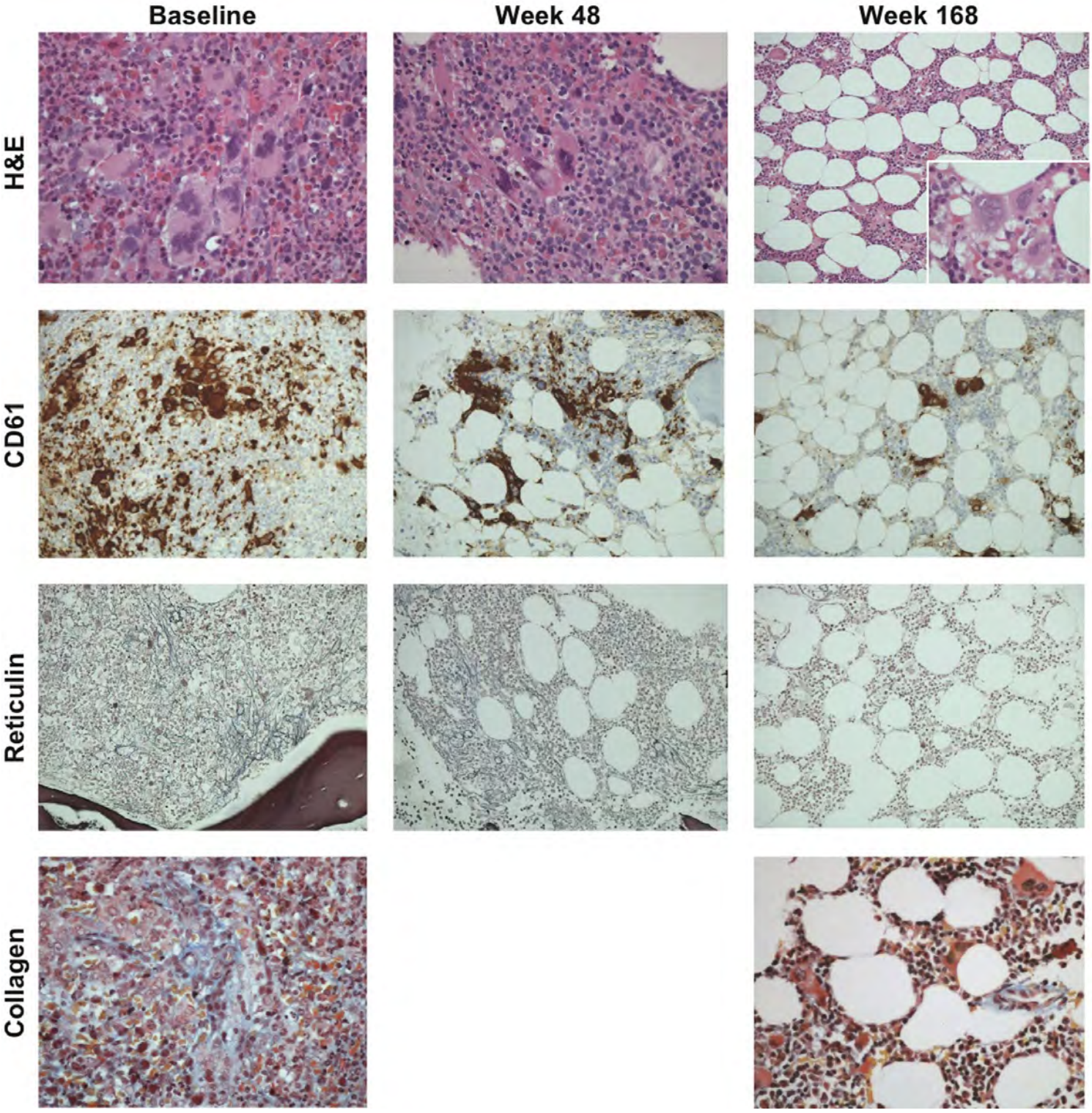
Figure 3. Percentage change from baseline in spleen volume over time.

Figure 4. Histologic characteristics of bone marrow at baseline and after 48 and 168 weeks of ruxolitinib treatment (a full description is provided in the text). H&E, hematoxylin and eosin.


Ruxolitinib dose (mg bid)
Spleen length (cm below the costal margin)
Absolute JAK2 V617F allele burden (%)
Study week
Ruxolitinib dose (mg bid)
Spleen length (cm below the costal margin)
JAK2 V617F allele burden (%)
0
5
10
15
20
25
30
0
10
20
30
40
50
60
70
80
90
100
0
12
24
36
48
60
72
84
96
108
120
132
144
156
168


Platelet levels (x 10⁹/L)
Hemoglobin levels (g/L)
250
200
150
100
50
0
0
12
24
36
48
60
72
84
96
108
120
132
144
156
168
Study week


Baseline spleen volume = 3390 cm3
Change from baseline in spleen volume (%)
10
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
0
12
24
36
48
60
72
84
96
108
120
132
144
156
168
Study week


Baseline
Week 48
Week 168
H&E
CD61
Reticulin
Collagen