Safety and efficacy of ruxolitinib in splanchnic vein thrombosis associated with myeloproliferative neoplasms

SAFETY AND EFFICACY OF RUXOLITINIB IN SPLANCHNIC VEIN THROMBOSIS ASSOCIATED WITH MYELOPROLIFERATIVE NEOPLASMS

Lisa Pieri [1,2], Chiara Paoli [1,2], Umberto Arena [3], Fabio Marra [4], Fabio Mori [5], Mery Zucchini [5], Stefano Colagrande [6], Alessandro Castellani [6], Arianna Masciulli [7], Vittorio Rosti [8], Valerio De Stefano [9], Silvia Betti [9], Guido Finazzi [7], Maria Luisa Ferrari [7], Elisa Rumi [10], Marco Ruggeri [11], Ilaria Nichele [11], Paola Guglielmelli [1,2], Rajmonda Fjerza [1,2], Carmela Mannarelli [1,2], Tiziana Fanelli [1,2,15], Lucia Merli [1,2], Giuditta Corbizi Fattori [1,2,15], Margherita Massa [12], Giuseppe Cimino [13], Alessandro Rambaldi [7], Giovanni Barosi [8], Mario Cazzola [10], Tiziano Barbui [14], Alessandro M. Vannucchi [1,2]

[1] CRIMM-Centro Ricerca e Innovazione per le Malattie Mieloproliferative, Azienda Ospedaliera-Universitaria Careggi, Florence, Italy;
[2] Department of Experimental and Clinical Medicine, University of Florence, and DenoThe Excellence Center, Florence, Italy;
[3] Internal Medicine and Hepatology, Azienda Ospedaliera-Universitaria Careggi, Florence, Italy;
[4] Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy;
[5] Department of Cardiology, Azienda Ospedaliera-Universitaria Careggi, Florence, Italy;
[6] 1Department of Experimental and Clinical Biomedical Sciences, Radiodiagnostic Unit N. 2, University of Florence - Azienda Ospedaliera-Universitaria Careggi, Florence, Italy;
7 Department of Hematology, Hospital Papa Giovanni XXIII, Bergamo, Italy;
8 Center for the Study of Myelofibrosis, Biotechnology Research Area, IRCCS Policlinico San Matteo Foundation, Pavia, Italy;
9Institute of Hematology, Catholic University, Rome, Italy;
10 Department of Hematology Oncology, IRCCS Policlinico San Matteo Foundation, Pavia, Italy;
11 Department of Hematology, San Bortolo Hospital, Vicenza, Italy;
12 Biotechnology Research Area, IRCCS Policlinico San Matteo Foundation, Pavia, Italy;
13 Department of Cellular Biotechnology and Hematology, University "La Sapienza", Rome, Italy;
14 Hospital Papa Giovanni XXIII and Research Foundation, Bergamo, Italy
15 Doctorate in genetic, oncology and clinical medicine, University of Siena, Siena, Italy

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1002/ajh.24614

Correspondence: Alessandro M. Vannucchi, CRIMM, Centro di Ricerca e Innovazione per le Malattie Mieloproliferative, Azienda Ospedaliera-Universitaria Careggi, Department of Experimental and Clinical Medicine, University of Florence, Viale Pieraccini, 6. Florence, 50134, Italy; e-mail: amvannucchi@unifi.it ; and Lisa Pieri CRIMM, Centro di Ricerca e Innovazione per le Malattie Mieloproliferative, Azienda Ospedaliera-Universitaria Careggi, Department of Experimental and Clinical Medicine, University of Florence, Viale Pieraccini, 6. Florence, 50134, Italy; e-mail: lisa.pieri@unifi.it .

Key words: myeloproliferative neoplasms; ruxolitinib; JAK2V617F; splanchnic vein thrombosis

Running title: Ruxolitinib in MPN with splanchnic vein thrombosis

Abstract word count: 246
Text word count : 3670
Figures/Tables : Figures: 2 Tables: 2
References : 32
Supplemental material: Included

ABSTRACT

Splanchnic vein thrombosis (SVT) is one of the vascular complications of myeloproliferative neoplasms (MPN). We designed a phase 2 clinical trial to evaluate safety and efficacy of ruxolitinib in reducing splenomegaly and improving disease-related symptoms in patients with MPN-associated SVT. Patients, diagnosed with myelofibrosis in 12 cases, polycythemia vera in 5 and essential thrombocythemia in 4, received ruxolitinib for 24 weeks in the core study period. Spleen volume was assessed by magnetic resonance imaging (MRI) and splanchnic vein circulation by echo-Doppler analysis. Nineteen patients carried *JAK2*V617F, one had *MPL*W515L and one *CALR*L367fs*46 mutation. Eighteen patients had spleno-portal-mesenteric thrombosis, 2 had Budd-Chiari syndrome, and one patient had both sites involved; 16 patients had esophageal varices. Ruxolitinib was well tolerated with hematological toxicities consistent with those of patients without SVT and no hemorrhagic adverse events were recorded. After 24 weeks of treatment, spleen volume reduction ≥35% by MRI was achieved by 6/21 (29%) patients, and a ≥50% spleen length reduction by palpation at any time up to week 24 was obtained by 13/21 (62%) patients. At week 72, 8 of the 13 (62%) patients maintained the spleen response by palpation. No significant effect of treatment on esophageal varices or in splanchnic circulation was observed. MPN-related symptoms, evaluated by MPN-SAF TSS questionnaire, improved significantly during the first 4 weeks and remained stable up to week 24. In conclusion, this trial shows that ruxolitinib is safe in patients with MPN-associated SVT, and effective in reducing spleen size and disease-related symptoms.

INTRODUCTION

Chronic Philadelphia negative myeloproliferative neoplasms (MPN) are hematologic disorders characterized by clonal expansion of a multipotent stem cell that maintains normal differentiation towards the different hematopoietic cell lineages but produces mature cells at higher levels. MPN include different clinical entities such as polycythemia vera (PV), essential thrombocythemia (ET) and myelofibrosis (MF); MF can arise as primary disease (primary myelofibrosis, PMF) or as the evolution of a previous PV and ET, known as post-PV/post-ET MF (PPV-MF, PET). These disorders share manifestations and complications; in particular, arterial and venous thrombosis represent severe and life-threatening clinical events, particularly in PV and ET [1]. Thrombosis in unusual sites, such as the splanchnic veins (SVT), is also typically associated with MPN [2-5]. The abnormalities of blood flow due to the occlusion of splanchnic vessels contribute to the development of splenomegaly, in addition to extramedullary hematopoiesis; splenomegaly represents one of the most compelling clinical manifestations in patients with MPN-associated SVT. Splanchnic vein thrombosis may cause portal hypertension that, beyond contributing further to splenomegaly, increases the risk of bleeding from varices [6-8].

Ruxolitinib is a JAK1/2 inhibitor approved for the treatment of splenomegaly or constitutional symptoms associated with myelofibrosis and for the treatment of adult patients with polycythemia vera resistant or intolerant to hydroxyurea. Ruxolitinib proved to be more efficacious in reducing the enlarged spleen and controlling MPN-related symptoms compared to either placebo (in MF, COMFORT-I) and best available therapy (in both MF and PV, COMFORT-II and RESPONSE study, respectively) [9-11]. Furthermore, results of long-term therapy in a phase II trial in patients with ET refractory or intolerant of hydroxyurea demonstrated efficacy of ruxolitinib in controlling thrombocytosis, reducing splenomegaly and improving symptoms [12].

We wanted to assess if therapy with ruxolitinib was safe and efficacious in reducing splenomegaly and improving symptomatology in patients with SVT associated with

MPN. We also evaluated whether the treatment induced a decrease of blood pressure in the splanchnic vessels thereby resulting in stabilization/reduction of pre-existing, and/or prevent the new formation, of esophageal varices. To this aim, we designed an investigator-initiated multicenter phase 2 study of ruxolitinib in patients with splenomegaly in the setting of SVT associated with MPN (SVT-RUXO trial, study code CINC424XIT01T, EudraCT number 2012-002253-30). We report here the final results of the study.

PATIENTS AND METHODS

PATIENT POPULATION

Study patient population was represented by adult subjects with SVT (including Budd-Chiari syndrome (BCS), mesenteric vein thrombosis, portal vein thrombosis, splenic vein thrombosis) in the setting of a diagnosis of PV, ET and PMF according to the 2008 WHO criteria [13], or PPV-MF and PET-MF according to the IWG-MRT criteria [14]. The SVT could have antedated or followed the diagnosis of MPN. Inclusion and exclusion criteria are reported in the online supplements.

STUDY DESIGN

Patients received ruxolitinib for 24 weeks in the core study period. Starting dose was 10 mg BID for patients diagnosed as PV, 25 mg BID for ET, 15 mg BID for MF if the baseline platelet count was >100 to $200x10^9$/L and 20 mg BID if $>200x10^9$/L. Patients who completed the core study period, with no major toxicity from the treatment and with evidence of clinically-significant improvement, were allowed to continue to receive the study drug in a protocol extension phase **(Supplementary figure 1).** Considering the concomitant use of vitamin K antagonist and/or antiplatelet agents, and the potential high risk of bleeding from varices, the cut off of thrombocytopenia for drug interruption was set at $75x10^9$/L instead of $50x10^9$/L, as in previous trials.

The primary study objective was to evaluate the proportion of patients achieving ≥50% reduction by palpation in spleen length from LCM at any visit and at week 24, or a ≥35% reduction in spleen volume (SV) by magnetic resonance imaging (MRI) or computed tomography (CT) at week 24. Secondary objectives, that were all

evaluated at week 24, included the following: safety of treatment; improvement in quality of life; modifications in the extension of the splanchnic vessel thrombosis; changes in hyperdynamic arterial circulation status; improvement of stiffness of hepatic and splenic parenchyma; changes in the status of esophageal varices; clinical and hematological response according to ELN criteria for PV and ET [15] and 2006-IWG-MRT [16] criteria for MF. In post-hoc analysis also the 2013-IWG-MRT criteria, not available at the time of protocol design, were used [17].

Exploratory evaluations included: changes in the level of J*AK2*V617F allele burden; analysis of the association of additional somatic mutations at baseline with response; changes in plasma inflammatory cytokine levels; quantification of circulating progenitor and mature endothelial cells (EPCs and CECs, respectively); changes in the expression profile of selected microRNAs in granulocytes; changes in MRI-based analysis of diffusion weighted imaging (DWI) of the spleen.

The study was approved by the Institutional Review Boards of the coordinating center in Florence (no. 43, June 11, 2012) and by participating institutions and was conducted in accordance with the Declaration of Helsinki. All patients provided written informed consent for the study and pharmacodynamic analysis.

The drug was provided free of charge by Novartis, that had no role in trial design or data analysis. Assessment and laboratory measurements and statistical analysis are described in details in the online supplements. To ensure maximal reproducibility, all these measurements were centralized except for esophagogastroduodenoscopy that was performed locally. The study was supported by AIRC, a no profit Italian organization for cancer research, in the framework of the AGIMM project (www.progettoagimm.it).

RESULTS

PATIENT CHARACTERISTICS AND DISPOSITION

Twenty-one patients were enrolled. The diagnosis was PMF in 8 patients (38.1%), PV in 5 (23.8%), ET in 4 (19.1%), PPV-MF in 3 (14.3%) and PET-MF in 1 (4.8%). Eighteen patients had spleno-portal-mesenteric thrombosis and two had BCS; one patient had

both sites involved. Seventeen patients had at least two vessels occluded while in 2 patients thrombosis involved only the portal and splenic vein, respectively. Sixteen patients (84%) had portal cavernoma and 17 (89%) had other porto-systemic collateral venous shunts, including splenorenal, esophagogastric, perisplenic, gallbladder, peripancreatic and abdominal wall shunts. Diagnosis of SVT occurred 80 months (median value, range 5-324) before ruxolitinib treatment. A *JAK2*V617F mutation was found in 90% of the patients, the median allele burden was 43% (range, 16-74); one patient each harbored a *MPL*W515L and a *CALR* type 1 mutation. The median length of the spleen from LCM was 10 cm, ranging from 5 to 21 cm; 4 patients (19%) also had palpable hepatomegaly. Fourteen patients had received hydroxyurea as cytoreductive treatment that was interrupted at least 7 days before ruxolitinib treatment. Ninety per cent of the patients were on anticoagulation, 1 on low-dose aspirin and 1 on both vitamin K antagonist and aspirin. Eleven patients were on beta-blockade therapy at study entry and remained at the same dosage at least up to w72 evaluations. No patients started beta-blockers during the study. The IPSS risk score of the 12 patients with MF was low in 4 (33.3%), intermediate-1 in 7 (58.3%) and intermediate-2 in 1 (8.3%).

Clinical and laboratory features are reported in **Table I**. At week 72 of follow-up, 18 patients (85.7%) were still on therapy. Two patients (9.5%) discontinued at week 24 due, respectively, to adverse events (asthenia and thrombocytopenia) and lack of clinical and/or hematological improvements.

The median ruxolitinib total daily dose at week 24 was 25 mg for MF, 18 mg for PV and 22.5 mg for ET.

SAFETY

Ruxolitinib was well tolerated, no serious adverse events (AE, recorded using National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) Version 4.03.) occurred. Hematologic toxicities up to week 24 included mainly thrombocytopenia, occurring in 52.4% of the patients (all grades) with grade 3 in 14.3%, and anemia, that occurred in 23.8% of cases (all grades) with grade 3 in 9.5%. Neutropenia occurred in two patients (9.5%), in both cases grade 3. No grade 4

hematologic toxicity was reported and no patients required red blood cell or platelet transfusions; hematologic AEs were managed with temporary withdrawal or dose reduction. Non-hematologic AEs up to week 24, all grade 1-2, consisted of mild infections, mainly upper respiratory tract, asthenia, abdominal symptoms and AST/ALT elevation; none of these AEs required dose withdrawal or dose reduction. A detailed report of AEs is presented in **Table II.** In particular, no bleeding episode occurred. There was one herpes zoster reactivation.

EFFICACY

At week 24 of treatment, a ≥50% spleen length reduction by palpation was obtained by 13 patients (61.9%) and a SV reduction ≥35% by imaging was achieved by 6 patients (29%) (**Figure 1A and B**). All patients showed evidence of some degree of SVR by MRI at week 24, ranging from -6% to -54% from baseline and 19/21 patients (90.5%) had also evidence of reduction of spleen length by palpation, ranging from -13% to -100% from baseline. Serial measurements of spleen length by palpation at 4-week intervals showed that a spleen response, ie reduction of spleen length ≥50%, was achieved by progressively more patients along with treatment duration: 42.9% (9/21) at week 4 and week 8, 47.6% (10/21) at week 12, 57.1% (12/21) at week 16 and 61.9% (13/21) at week 20. The median reduction from baseline is shown on **Figure 1C**.

Evaluation of the status of splanchnic vein circulation by echo-Doppler analysis at 24 weeks revealed that the extension of venous thrombosis had remained stable compared to baseline. The resistive index of intraparenchymal splenic and hepatic artery and the pulsatility index of mesenteric artery did not change appreciably during therapy: values at screening and week 24 were 0.61 (range 0.52-0.79) versus 0.63 (0.5-0.8) (p=0.7), 0.66 (0.53-6.0) versus 0.66 (0.5-0.8)(p=0.36) and 2.2 (0.9-5.0) versus 2.4 (0.8-7.0)(p=0.96), respectively.

Spleen stiffness by fibroscan [18, 19] could be evaluated only in 4 patients due to baseline levels that were higher than the instrument upper limit of detection in the remaining 17 patients. In all four evaluable cases, spleen stiffness reduced from a median of 55.85 Kpa (range 34.3-75.0) to 42.5 Kpa (range 26.3-72.0), with a

median reduction of 24%. This observation was consistent with report in MF patients without SVT who were treated with ruxolitinib [20]. Measures of hepatic stiffness at baseline were within normal range for all patients and no changes occurred at w24 (not shown in detail).

At baseline 5 patients did not present esophageal varices, 9 had varices grade 1 (F1) and 7 had grade 2 (F2). At week 24, none of the 5 patients without varices at baseline had evidence of de-novo formation. Eight of nine (88.9%) patients with F1 remained stable and one worsened to grade 2 (11.1%), while of the 7 patients with varices F2, 4 remained stable (57.1%), 2 (28.6%) worsened to grade 3 and 1 improved to F1 following a banding performed after 14 weeks while on ruxolitinib but pre-planned before enrolment. No patient experienced upper gastrointestinal bleeding, and none underwent banding except for the pre-planned procedure.

Cardiac assessment at week 24 showed a reduction of heart rate from a median value of 76 bpm (range 55-110) at baseline to 63 bpm (range 45-90) (*P*=.001), leading to a median reduction of cardiac output (CO) of 16.3% (range -41.8 to 38.5%; *P*=.031). The increase of body mass index (BMI) associated with ruxolitinib therapy (from a median of 21.8, range 18.3-34.9, to 22.2, range 20.6-39.3, *P*=.025) led to a reduction of cardiac index (CI) of -17.9% from baseline (range -43.2 to 38.5; *P*=.024). No changes in indexes of systolic and diastolic function were noted. Reduction of CO and CI were positively correlated with percentage reduction of spleen volume (r^2=0.28, CI 0.18-1.7, p=0.018; and r^2=0.31, CI 0.22-1.8, p=0.016, respectively).

Quality of life and symptoms related to MPN were assessed using the MPN-SAF-TSS questionnaire [21] at baseline and at each monthly visit up to week 24. At baseline, PV was the most symptomatic disease with a median score of 35, followed by ET with 33 and MF with 21. The most commonly reported symptom was fatigue (21/21 patients), followed by itching (18/21), early satiety (17/21), abdominal discomfort (16/21) and night sweats (15/21). The median total symptom score for all patients decreased from 23 (range 3-54) at baseline to 12 (range 3-49) at week 24 (*P*<.001). For patients with PV, the median reduction was from 35 (range 5-50) at baseline to 21 (4-36, *P*=.06) at week 24; for patients with ET it was from 33 (19-45) to

13 (7-20, *P*=.12) and for MF from 21 (3-54) to 11 (3-49, *P*=.02) **(Figure 2A, B).** Reductions of MPN-SAF TSS score occurred since the first 4 weeks of treatment and remained substantially stable from week 8 to week 24 (**Figure 2B**).

Clinicohematological response for patients with MF was assigned according to the 2006 IWG-MRT criteria, originally considered in this trial design. Four of 12 patients (33%) obtained clinical improvement (CI) and 8 stable disease (SD) (67%); according to the 2013-IWG-MRT criteria there were 3 CI (25%, for symptoms and/or spleen response) and 9 SD (75%). Response for PV and ET patients was evaluated according to the ELN criteria. Of the 9 ET/PV patients, 8 (89%) had partial response and 1 ET patient had a complete response.

EXPLORATORY ENDPOINTS.

Nineteen patients carried the *JAK2*V617F mutation, with a median allele burden at baseline of 43%, range 16-74. At week 24, the median allele burden was 40%, range 14-67, with a reduction of 11% consistent with other studies [9, 22-25].

Of the 6 cytokines whose plasma levels were evaluated after 4 weeks of treatment (IL-8, IL-12, sTNF-RII, hs-CRP, GM-CSF and VEGF; see Supplemental information) two showed significant reductions: VEGF from a median values of 14 ng/mL (range 7-35) to 9 ng/mL(range 5-30)(*P*=.02) and sTNFrII from 12 ng/mL (range 2-76) to 8 ng/mL (range 3-50) (*P*=.04).

No statistically significant variations were observed in the plasma levels of the 13 miRNA evaluated after 4 weeks of treatment (see Supplemental information for details).

The absolute number of circulating endothelial progenitor cells represented by different subsets including $Syto^{+}CD34^{+}VEGFR\text{-}2^{+}$, $Syto^{+}CD45\text{-}CD34^{+}CD133^{+}VEGFR\text{-}2^{+}$, and $Syto^{+}CD45^{dim}CD34^{+}VEGFR\text{-}2^{+}$ cells, was significantly lower (*P*=.0005, *P*=.027, and *P*=.0009, respectively) at week 24 of treatment in comparison to baseline levels. On the contrary, no significant change was found in the frequency of circulating endothelial cells, identified as $Syto^{+}CD45^{-}CD31^{+}CD146^{+}$ cells **(Supplementary table III)**.

The analysis of Diffusion Weighted Imaging (DWI) of the spleen parenchyma expressed as the Apparent Diffusion Coefficient (ADC), was performed at baseline and week 24 by MRI [26]; information at both baseline and week 24 were available in 17/21 patients. We observed that ADC values overall decreased by 21% at week 24, from a median of 1491 mm^2/s (range 1020-1835) at baseline to 1181 mm^2/s (990-1503) (*P*=.002).

FOLLOW UP AT WEEK 72.

Eighteen patients were still on treatment. Other than the two patients who discontinued at w24, the third continued ruxolitinib after shifting to commercial use (1/21, 4.8%). At week 72, spleen response was evaluated only by palpation; of the 13 patients that had obtained a ≥ 50% spleen length reduction at week 24, eight (61.5%) maintained the spleen response. One patient (4.8%) obtained the spleen response after week 24 (at week 48) and 7/21 (33.3%) never achieved it. Median reduction at week 72 was -50.5% (range 0-100). None of the four patients without esophageal varices at baseline who were still on treatment at week 72 and performed esophagogastroduodenoscopy had evidence of de novo varices. Of the 8 patients with F1 varices, 6 (75%) remain stable and 2 (25%) worsened to grade 2; one of them performed banding procedure. All 5 patients with F3 at baseline remained stable at week 72. Overall, 85% of the patients showed stability of the varices grade compared with baseline.

Safety profile was comparable to 24 weeks, with a total of 11 new, grade 1-2, adverse events consisting of ascites, muscle cramps and weight gain (**Supplementary table IV**), confirming the ruxolitinib safe risk profile in longer follow up [27]. One gastrointestinal bleeding episode occurred in one patient. After dose adjustment, eventually required because of hematological toxicity, the median total daily dose at week 72 was 19.1 mg for MF, 16 mg for PV and 28.3 for ET.

*JAK2*V617F allele burden was measured in 13 patients: median allele burden was 41% (range 10-74) with a reduction from baseline of 10.3% (range 0-38), similar to w24.

DISCUSSION

MPNs are a frequent underlying cause of SVT. [4] In these patients, in addition to the myeloproliferative process, the portal hypertension contributes to pathogenesis of splenomegaly and eventually induces hypersplenism that contributes to cytopenias. In most patients, blood counts are often within or below normal range, yet the thrombosis itself qualifies patients as at "high risk", therefore cytoreduction is indicated [28] in addition to vitamin K antagonist and/or antiplatelet agents. [3] Such features make MPN patients with an associated SVT frailer than those without SVT, particularly because of high risk of severe bleeding from sites such as the esophageal varices. Ruxolitinib demonstrated superiority to placebo (COMFORT-I) and best available therapy (COMFORT-II) in patients with primary and secondary forms of myelofibrosis as regards volume reduction of the enlarged spleen and symptomatic improvement; also, in patients with PV refractory or resistant to hydroxyurea, ruxolitinib was superior to best available therapy in a combined primary endpoint of hematocrit control and spleen volume reduction. Information in the setting of SVT associated with MPN are scanty. A case report described the improvement of esophageal varices secondary to portal hypertension in a patient with MF who had received ruxolitinib [29]. On the opposite, none of three patients with MPN and portal hypertension due to SVT, who were treated with ruxolitinib in another study, showed meaningful change in the grade of esophageal varices [30].

The leading question we wanted to address with this study concerned the efficacy and safety of ruxolitinib, particularly regarding the hematological toxicity and bleeding episodes, in this settings of patients with MPN that were poorly represented in previous controlled studies. Results indicated that SV reduction ≥35% by imaging at week 24 of treatment was obtained in 29% of patients, a figure that is similar to COMFORT-II (32%) at week 24 [10] and to COMFORT-II at week 48 (28%) [9]. A comparable figure of spleen volume reduction ≥35% (38.2%) was reported at week 32 in patients with PV enrolled in the RESPONSE study [11]. Similar to those studies, we also found that the proportion of patients with spleen length reduction increased progressively along with treatment duration, suggesting that some responses might occur later than 24 weeks. Also the symptomatic improvement

observed in SVT patients was consistent with previous experiences. Hematologic and extra-hematologic toxicities were similar to those observed in controlled studies, in particular we did not recorded any bleeding episode, even if ruxolitinib was interrupted at a higher platelet count threshold ($75x10^9$/L) than used in previous studies ($100x10^9$/L). According to the inclusion/exclusion criteria, only patients with platelets higher than $100x19^9$/L and no recent history of bleeding were allowed to enter the trial; therefore some selection of the patients might have occurred since up to 25% of patients with SVT and MPN are thrombocytopenic.

Results of this trial do not support that treatment with ruxolitinib produces meaningful improvements in the status of esophageal varices during treatment, but might suggest that treatment was associated with substantial stabilization of varices grade at week 72, as observed in 85% of the patients; furthermore there was only one bleeding episode. At this regard, a recent study in chronic noncirrhotic, nontumoral portal vein thrombosis reported an incidence of newly developed varices of 2% at 1 year and 22% at 3 years, and probability of worsening of existing esophageal varices of 13% and 40% at 1 and 3 years, respectively. The same study reported a probability of bleeding in patients with large esophageal varices of 9% and 20% at 1 and 3 years. [31] Clearly, longer follow up and larger number of patients are needed before reaching any firm conclusion about impact of ruxolitinib on varices. Other parameters reflecting the functional status of portal hypertension, such as the resistive indexes of the intraparenchymal arteries of the spleen and liver, remained unchanged during treatment; however, while these measures have been extensively studied in patients with portal hypertension due to cirrhosis, the lack of data in patients with a pre-hepatic portal hypertension as those with SVT-MPN makes uncertain a thorough interpretation of results. We observed that patients with SVT and MPN enrolled in this study presented normal values of liver stiffness, that is different from portal hypertension associated with cirrhosis [32]. On the contrary, spleen stiffness was markedly increased in all SVT-MPN patients, actually exceeding the upper limit of measurement with conventional methods in most of them; however, in all the 4 patients in which such measurement was feasible, a marked reduction of spleen stiffness was documented, suggesting that reduction of spleen volume induced by ruxolitinib might be in part associated with changes in the

spleen parenchyma. This hypothesis is supported further by the observed changes in the diffusion weighted imaging (DWI) of the spleen. In this regard, we found that ADC, a parameter that estimates the water molecular average motion in a tissue, was significantly reduced by ruxolitinib at 24 week, suggesting a shrinking of the interstitial tissue rather than a reduction of cellular components of the splenic parenchyma. The involvement of the vascular compartment in the mechanistic response of spleen volume induced by ruxolitinib is further supported by the observed reduction of circulating EPCs and the plasma levels of VEGF [23].

We also described the occurrence of a significant reduction of heart rate during ruxolitinib therapy associated with changes in cardiac output. We hypothesize that the lowering of heart rate might be related to the reduction of spleen volume and of some proinflammatory cytokines, such as sTNFrII. Moreover, the reduction of cardiac output, combined with an increase body surface area due to weight gain, resulted in a meaningful decrease of the cardiac index. Overall, such changes in systemic circulation might be potentially of benefit in at least some SVT-MPN patients with abnormal cardiovascular function due to the underlying thrombotic event.

In conclusion, results of this phase 2 trial demonstrated that ruxolitinib is safe in patients with MPN associated to SVT and is effective in reducing spleen size and disease-related symptoms.

ACKNOWLEDGEMENTS

This work was supported by a special grant from Associazione Italiana per la Ricerca sul Cancro-“AIRC 5 per Mille”- to AGIMM, “AIRC-Gruppo Italiano Malattie Mieloproliferative” (#1005) at www.progettoagimm.it. (A.M.V.)

Novartis provided the drug free of cost and contributed with a research grant to University of Florence. (A.M.V.)

AUTHORSHIP

Contribution: L.P. designed research, performed research, analyzed data, and wrote the paper; C.P. and A.M. collected and analyzed data; U.A., F.Marra, F.Mori, M.Z.,

S.C., A.C. designed research, performed research, contributed to analytical tools. V.R., V.D.S., S.B., G.F., M.L.F., E.R., M.R., I.N., P.G., R.F., L.M., G.C., A.R., G.B., M.C., T.B. collected data; C.M., T.F., G.C.F., M.M. performed research and analyzed data; A.M.V. designed research, performed research, analyzed data, and wrote the paper.

Conflict-of-interest dislosure: A.M.V: Novartis, advisory board, speaker fee, institutional grant support. A.R.: consultancy for Sigmatau Research Switzerland SA and honoraria from Pierre Fabre, Novartis, Hoffmann La Roche. T.B.: Speakers Bureau for Novartis. Other authors declare no conflicts of interest.

BIBLIOGRAPHY

1. Vannucchi AM, Guglielmelli P, Tefferi A. Advances in understanding and management of myeloproliferative neoplasms. CA: a cancer journal for clinicians 2009;59:171-191.
2. Sekhar M, McVinnie K, Burroughs AK. Splanchnic vein thrombosis in myeloproliferative neoplasms. British journal of haematology 2013;162:730-747.
3. De Stefano V, Qi X, Betti S, et al. Splanchnic vein thrombosis and myeloproliferative neoplasms: molecular-driven diagnosis and long-term treatment. Thrombosis and haemostasis 2015;115.
4. Smalberg JH, Arends LR, Valla DC, et al. Myeloproliferative neoplasms in Budd-Chiari syndrome and portal vein thrombosis: a meta-analysis. Blood 2012;120:4921-4928.
5. Primignani M, Barosi G, Bergamaschi G, et al. Role of the JAK2 mutation in the diagnosis of chronic myeloproliferative disorders in splanchnic vein thrombosis. Hepatology 2006;44:1528-1534.
6. De Stefano V, Martinelli I. Splanchnic vein thrombosis: clinical presentation, risk factors and treatment. Internal and emergency medicine 2010;5:487-494.
7. Kiladjian JJ, Cervantes F, Leebeek FW, et al. The impact of JAK2 and MPL mutations on diagnosis and prognosis of splanchnic vein thrombosis: a report on 241 cases. Blood 2008;111:4922-4929.
8. Donadini MP, Dentali F, Ageno W. Splanchnic vein thrombosis: new risk factors and management. Thrombosis research 2012;129 Suppl 1:S93-96.
9. Vannucchi AM, Kiladjian JJ, Griesshammer M, et al. Ruxolitinib versus standard therapy for the treatment of polycythemia vera. N Engl J Med 2015;372:426-435.
10. Harrison C, Kiladjian JJ, Al-Ali HK, et al. JAK inhibition with ruxolitinib versus best available therapy for myelofibrosis. N Engl J Med 2012;366:787-798.
11. Verstovsek S, Mesa RA, Gotlib J, et al. A double-blind, placebo-controlled trial of ruxolitinib for myelofibrosis. The New England journal of medicine 2012;366:799-807.
12. Verstovsek S, Passamonti F, Rambaldi A, et al. Long-Term Results from a Phase II Open-Label Study of Ruxolitinib in Patients with Essential Thrombocythemia Refractory to or Intolerant of Hydroxyurea. Blood 2014;124:1847-1847.
13. Tefferi A, Vardiman JW. Classification and diagnosis of myeloproliferative neoplasms: the 2008 World Health Organization criteria and point-of-care diagnostic algorithms. Leukemia 2008;22:14-22.
14. Cervantes F PF, Barosi G. Life expectancy and prognostic factors in the classic BCR/ABL-negative myeloproliferative disorders. Leukemia 2008;22:905-914.
15. Barosi G BG, Finazzi G, Griesshammer M, Harrison C, Hasselbalch HC, Kiladjian JJ, Lengfelder E, McMullin MF, Passamonti F, Reilly JT, Vannucchi AM, Barbui T. Response criteria for essential thrombocythemia and polycythemia

vera: result of a European LeukemiaNet (ELN) consensus conference. Blood 2009;113:4829-4833.
16. Tefferi A BG, Mesa RA, Cervantes F, Deeg HJ, Reilly JT, Verstovsek S, Dupriez B, Silver RT, Odenike O, Cortes J, Wadleigh M, Solberg LA Jr, Camoriano JK, Gisslinger H, Noel P, Thiele J, Vardiman JW, Hoffman R, Cross NC, Gilliland DG, Kantarjian H; IWG for Myelofibrosis Research and Treatment (IWG-MRT). International Working Group (IWG) consensus criteria for treatment response in myelofibrosis with myeloid metaplasia, for the IWG for Myelofibrosis Research and Treatment (IWG-MRT). Blood 2006;108:1497-1503.
17. Tefferi A, Cervantes F, Mesa R, et al. Revised response criteria for myelofibrosis: International Working Group-Myeloproliferative Neoplasms Research and Treatment (IWG-MRT) and European LeukemiaNet (ELN) consensus report. Blood 2013;122:1395-1398.
18. Colecchia A, Montrone L, Scaioli E, et al. Measurement of spleen stiffness to evaluate portal hypertension and the presence of esophageal varices in patients with HCV-related cirrhosis. Gastroenterology 2012;143:646-654.
19. Pinzani M, Vizzutti F, Arena U, et al. Technology Insight: noninvasive assessment of liver fibrosis by biochemical scores and elastography. Nature clinical practice Gastroenterology & hepatology 2008;5:95-106.
20. Iurlo A, Cattaneo D, Giunta M, et al. Transient elastography spleen stiffness measurements in primary myelofibrosis patients: a pilot study in a single centre. British journal of haematology 2015;170:890-892.
21. Scherber R, Dueck AC, Johansson P, et al. The Myeloproliferative Neoplasm Symptom Assessment Form (MPN-SAF): international prospective validation and reliability trial in 402 patients. Blood 2011;118:401-408.
22. Deininger M, Radich J, Burn TC, et al. The effect of long-term ruxolitinib treatment on JAK2p.V617F allele burden in patients with myelofibrosis. Blood 2015;126:1551-1554.
23. Verstovsek S, Passamonti F, Rambaldi A, et al. A phase 2 study of ruxolitinib, an oral JAK1 and JAK2 Inhibitor, in patients with advanced polycythemia vera who are refractory or intolerant to hydroxyurea. Cancer 2014;120:513-520.
24. Vannucchi AM, Passamonti F, Al-Ali HK, et al. Reductions in JAK2 V617F Allele Burden with Ruxolitinib Treatment in Comfort-II, a Phase 3 Study Comparing the Safety and Efficacy of Ruxolitinib with Best Available Therapy (BAT). Blood 2012;120:802-802.
25. Pieri L, Pancrazzi A, Pacilli A, et al. JAK2V617F complete molecular remission in polycythemia vera/essential thrombocythemia patients treated with ruxolitinib. Blood 2015;125:3352-3353.
26. Colagrande S, Carbone SF, Carusi LM, et al. Magnetic resonance diffusion-weighted imaging: extraneurological applications. La Radiologia medica 2006;111:392-419.
27. Harrison CN, Vannucchi AM, Kiladjian JJ, et al. Long-term findings from COMFORT-II, a phase 3 study of ruxolitinib vs best available therapy for myelofibrosis. Leukemia 2016;30:1701-1707.
28. Barbui T, Barosi G, Birgegard G, et al. Philadelphia-negative classical myeloproliferative neoplasms: critical concepts and management recommendations from European LeukemiaNet. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2011;29:761-770.

29. Koschmieder S, Koppelle A, Seifert H. Ruxolitinib for myelofibrosis. N Engl J Med 2012;366:2031-2032; author reply 2032-2034.
30. Yan M, Geyer H, Mesa R, et al. Clinical features of patients with Philadelphia-negative myeloproliferative neoplasms complicated by portal hypertension. Clinical lymphoma, myeloma & leukemia 2015;15:e1-5.
31. Noronha Ferreira C, Seijo S, Plessier A, et al. Natural history and management of esophagogastric varices in chronic noncirrhotic, nontumoral portal vein thrombosis. Hepatology 2016;63:1640-1650.
32. Vizzutti F, Arena U, Romanelli RG, et al. Liver stiffness measurement predicts severe portal hypertension in patients with HCV-related cirrhosis. Hepatology 2007;45:1290-1297.

FIGURE LEGENDS.

Figure 1. Spleen reduction during treatment. (**A**) Spleen length reduction measured by palpation and (**B**) spleen volume reduction measured by imaging at week 24. **C**: Spleen length reduction by palpation measured every 4 weeks from baseline to week 24; the median reduction from baseline at each time point is shown on the bottom.

Figure 2. Symptoms assessment with the MPN-SAF TSS questionnaire. **A.** Percentage change from baseline to week 24. **B.** Total score at each time point with median reduction from baseline is shown at the bottom.

Table I. Patients' characteristics at baseline.

	Patient no. (%)
Males	9 (42.9)
Age at enrolment Median (min-max)	49.8 (35.4-70.5)
Diagnosis Primary Myelofibrosis PV ET Post PV-Myelofibrosis Post ET-Myelofibrosis	 8 (38.1) 5 (23.8) 4 (19.0) 3 (14.3) 1 (4.8)
Budd-Chiari syndrome Spleno-porto-mesenteric thrombosis Budd-Chiari and spleno-porto-mesenteric thrombosis	2 (9.5) 18 (85.7) 1 (4.8)
Patients with JAK2V617F mutation VF allele burden <50% VF allele burden >50% Median (range, min-max) mutant alleles burden	19 (90.5) 12 (63.2) 7 (36.8) 43 (16.0-74.0)
Patients with MPLW515L mutation	1 (4.8)
Patients with CALR mutation	1 (4.8)
ECOG 0, no. (%) 1, no. (%) 2, no. (%)	 16 (76.2) 3 (14.3) 2 (9.5)
LDH, U/L, median (min-max)	248 (154-879)
Hemoglobin, gr/dL, median (min-max)	12.9 (9.4-16.7)
Platelet count, x10^9/L, median (min-max)	212 (100-389)
White blood cell count, x10^9/L, median (min-max)	7.3 (1.8-16.4)
Splenomegaly, cm below LCM, median (range)	10 (5-21)
Patients with palpable hepatomegaly	4 (19.1)
History of hemorrhagic events	1 (4.8)
Patients on vitamin K antagonist therapy	19 (90.4)
Patients in antiplatelet treatment	1 (4.8)
Patients on both vitamin K antagonist and antiplatelet treatment	1 (4.8)
Patients on beta-blockade treatment	11 (52.4)
Patients receiving cytostatic treatment before enrollment	14 (66.7)

Table II. Adverse events occurred in more than 10% of patients up to week 24, irrespective of causality.

Adverse event definition	Grade*	Number of events	Number of patients	Percentage of patients (%)
Platelet count decreased	all	21	11	52.4
	1,2	17	11	52.4
	3,4	4	3	14.3
Anemia	all	12	5	23.8
	1,2	10	5	23.8
	3,4	2	2	9.5
Neutropenia	all	4	2	9.5
	1,2	2	2	9.5
	3,4	2	2	9.5
Asthenia	all	4	4	19.0
	1,2	4	4	19.0
	3,4	0	0	0.0
Fever/Flu like symptoms	all	6	4	19.0
	1,2	6	4	19.0
	3,4	0	0	0.0
Upper airways infection	all	4	3	14.3
	1,2	4	3	14.3
	3,4	0	0	0.0
AST or ALT increase	all	11	6	28.6
	1,2	11	6	28.6
	3,4	0	0	0.0
Diarrhea	all	3	3	14.3
	1,2	3	3	14.3
	3,4	0	0	0.0
Abdominal pain	all	3	2	9,5
	1,2	3	2	9,5
	3,4	0	0	0,0

* According to CTC AE 4.03.

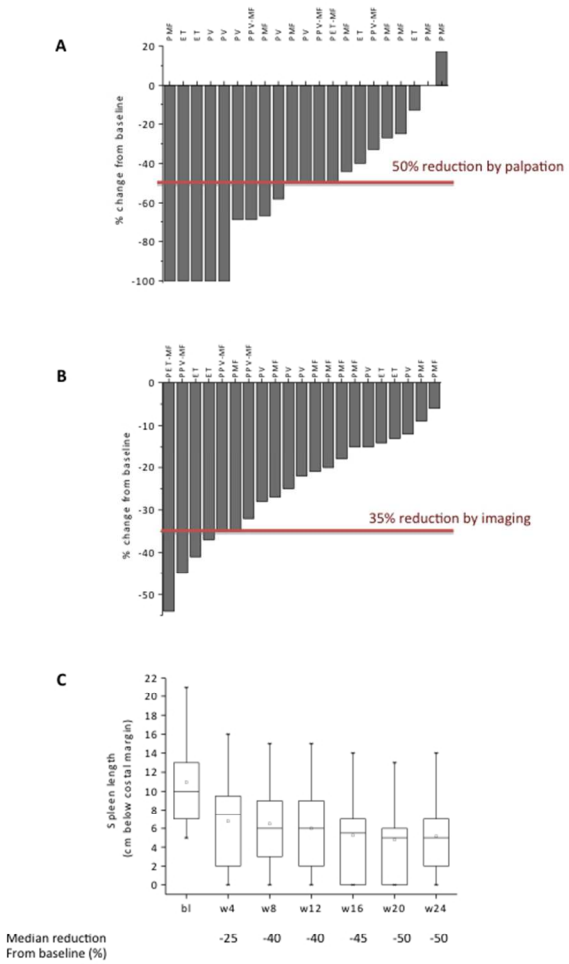

Figure 1. Spleen reduction during treatment. (A) Spleen length reduction measured by palpation and (B) spleen volume reduction measured by imaging at week 24. C: Spleen length reduction by palpation measured every 4 weeks from baseline to week 24; the median reduction from baseline at each time point is shown on the bottom.

254x366mm (72 x 72 DPI)

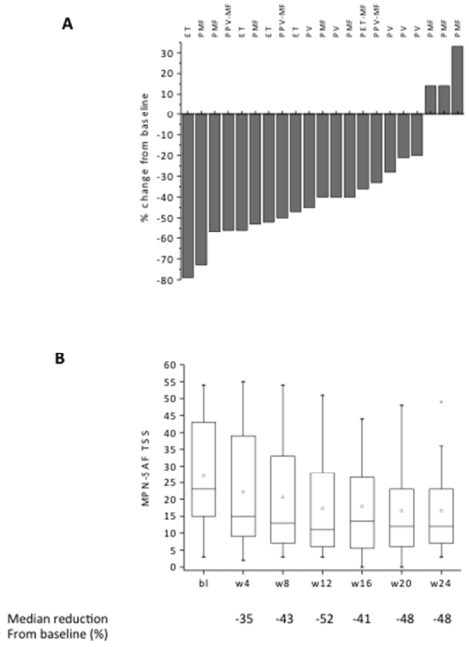

Figure 2. Symptoms assessment with the MPN-SAF TSS questionnaire. A. Percentage change from baseline to week 24. B. Total score at each time point with median reduction from baseline is shown at the bottom.

254x366mm (72 x 72 DPI)