Ruxolitinib rechallenge in resistant or intolerant patients with myelofibrosis: Frequency, therapeutic effects, and impact on outcome

Ruxolitinib Rechallenge in Resistant or Intolerant Patients with Myelofibrosis: Frequency, Therapeutic Effects, and Impact on Outcome

Francesca Palandri[1], Mario Tiribelli[2], Massimo Breccia[3], Daniela Bartoletti[1], Elena M. Elli[4], Giulia Benevolo[5], Bruno Martino[6], Francesco Cavazzini[7], Alessia Tieghi[8], Alessandra Iurlo[9], Elisabetta Abruzzese[10], Novella Pugliese[11], Gianni Binotto[12], Giovanni Caocci[13], Giuseppe Auteri[1], Daniele Cattaneo[9], Malgorzata M. Trawinska[10], Rossella Stella[2], Luigi Scaffidi[14], Nicola Polverelli[15], Giorgia Micucci[16], Elena Masselli[17], Costanza Bosi[18], Florian H. Heidel[19], Roberto Latagliata[20], Fabrizio Pane[11], Antonio Cuneo[7], Mauro Krampera[14], G. Semenzato[12], Roberto M. Lemoli[21], Michele Cavo[1], Nicola Vianelli[1], Massimiliano Bonifacio*[14] , Giuseppe A. Palumbo*[22]

[1]IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy; [2]Division of Hematology and BMT, Azienda Sanitaria Universitaria Integrata di Udine, Italy; [3]Division of Cellular Biotechnologies and Hematology, University Sapienza, Rome, Italy; [4]Hematology Division, San Gerardo Hospital, ASST Monza, Italy; [5]Division of Hematology, Città della Salute e della Scienza Hospital, Torino, Italy; [6]Division of Hematology, Azienda Ospedaliera 'Bianchi Melacrino Morelli', Reggio Calabria, Italy; [7]Division of Hematology, University of Ferrara, Italy; [8]Department of Hematology, Azienda USL - IRCCS di Reggio Emilia, Reggio Emilia, Italy; [9]Hematology Division, Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy; [10]Division of Hematology, Ospedale S. Eugenio, Rome, Italy; [11]Department of Clinical Medicine and Surgery, Federico II University Medical School, Naples, Italy; [12]Unit of Hematology and Clinical Immunology, University of Padova, Padova, Italy; [13]Ematologia, Ospedale Businco, Università degli studi di Cagliari, Cagliari, Italy; [14]Section of Hematology, University of Verona, Verona, Italy; [15]Unit of Blood Diseases and Stem Cell Transplantation, ASST Spedali Civili di Brescia, Brescia, Italy; [16]Hematology and Stem Cell Transplant Center, AORMN Hospital, Pesaro, Italy; [17]Haematology and BMT Centre, Azienda Ospedaliero-Universitaria di Parma, Parma, Italy; [18]Division of Hematology, AUSL di Piacenza, Piacenza, Italy; [19]Hematology and Oncology, Friedrich-Schiller-University Medical Center, Jena, Germany; [20]Hematology Unit, Ospedale Belcolle, Viterbo, Italy; [21]Clinic of Hematology, Department of Internal Medicine (DiMI), IRCCS AOU San Martino-IST, Genova, Italy; [22]Department of Scienze Mediche, Chirurgiche e Tecnologie Avanzate “G.F. Ingrassia”, University of Catania, Italy.

*These Authors equally contributed to this manuscript

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Corresponding Author

Francesca Palandri, MD, PhD

Institute of Hematology “L. and A. Seràgnoli”, Azienda Ospedaliero-Universitaria di Bologna,

IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy

francesca.palandri@unibo.it

Abstract

Background: After ruxolitinib discontinuation, the outcome of patients with myelofibrosis (MF) is poor with scarce therapeutic possibilities.

Methods: We have performed a sub-analysis of the RUX-MF observational retrospective study, that includes 703 MF patients treated with ruxolitinib, to investigate: 1) frequency and reasons for ruxolitinib rechallenge; 2) therapeutic effects of rechallenge; 3) impact of rechallenge on overall survival (OS),

Results: A total of 219 (31.2%) discontinued ruxolitinib for ≥14 days and survived for ≥30 days. In 60 (27.4%) patients, ruxolitinib was re-challenged for ≥14 days (RUX-again), while 159 (72.6%) patients discontinued ruxolitinib permanently (RUX-stop). Baseline characteristics of the two cohorts were comparable, but discontinuation due to lack/loss of spleen response was lower in RUX-again patients ($p=0.004$). Compared to disease status at first ruxolitinib stop, at restart there was a significant increase of patients with large splenomegaly ($p<0.001$) and high TSS ($p<0.001$). During the rechallenge, 44.6% and 48.3% of patients improved spleen and symptoms, and there was a significant increase in patients with TSS reduction ($p=0.01$); the use of ruxolitinib dose >10mg BID was associated with spleen ($p=0.05$) and symptoms ($p=0.02$) improvements. At 1 and 2 years, 33.3% and 48.3% of RUX-again patients had permanently discontinued ruxolitinib, respectively. Median overall survival was 27.9 months and was significantly longer in RUX-again patients ($p=0.004$).

Conclusion: Ruxolitinib rechallenge was mainly used in intolerant patients, with clinical improvements and possible survival advantage in many cases, but with a substantial rate of permanent discontinuations. Ruxolitinib rechallenge should be balanced against newer therapeutic possibilities.

Introduction

Ruxolitinib is the first *JAK1/JAK2* inhibitor approved for the treatment of splenomegaly and symptoms related to myelofibrosis (MF) and has demonstrated significant efficacy in most patients, with improvement of quality of life and overall survival in responding patients[1,2]. Nevertheless, some patients cannot tolerate ruxolitinib, and many do not achieve or lose the response over time, leading to ruxolitinib discontinuation in around 50% of patients at 3 years[3,4]. After ruxolitinib discontinuation, the outcome is poor with scarce therapeutic possibilities, including palliation, investigational agents, allogeneic stem cell transplantation (ASCT), and splenectomy[4-7]. Other *JAK*-inhibitors have also been studied in MF over the last several years[8]. Among them, fedratinib has recently received FDA approval for use in patients intolerant of or resistant to ruxolitinib, with a response rate around 30%, regardless of the reason for ruxolitinib discontinuation[9]. Many other new agents, alone or in combination with ruxolitinib, are currently under investigation, particularly second-generation *JAK2*-inhibitors (eg, momelotinib and pacritinib)[10,11], telomerase inhibitor (eg, imetelstat)[12], BET inhibitor (eg, CPI-0610)[13], PI3/AKT inhibitors (eg, buparlisib)[14], LSD1 inhibitor (bomedemstat)[15], BCL-2/BCL-X inhibitors (eg, navitoclax)[16].

Beyond new drugs, a retrospective case series including 13 patients has suggested that patients may respond to a rechallenge of ruxolitinib after a first drug stop[17]. This therapeutic strategy may be attractive in routine clinical practice, as it is simple to implement and may include the frailest patients who cannot be enrolled in investigational clinical studies. However, it is not known how frequently, and for what reasons, ruxolitinib rechallenge is used in real-life practice, what its clinical effects are, and whether rechallenge may affect the outcome.

To answer these questions, we have performed a sub-analysis of an observational retrospective study (RUX-MF) that was promoted by the Institute of Hematology “L. and A. Seràgnoli” in Bologna, Italy.

Material and methods

Patients and study design

The RUX-MF observational retrospective study involves 703 consecutive MF patients treated with ruxolitinib in 22 academic hematology centres that are dedicated to the treatment of MF. The list of the participating centres is available in the **Appendix**. All centres were asked to report, in an electronic case report form (e-CRF), their consecutive MF patients who received ruxolitinib according to standard clinical practice. The total number of medical files was reported by each Centre by data input into an electronic database developed to record all study data after de-identification of the patients with an alphanumeric code to protect personal privacy. Data collected included patient demographics, comorbidities, medications, clinical/laboratory tests at diagnosis and during follow-up, date of ruxolitinib start and stop, type of MF therapies prior and after ruxolitinib, duration of ruxolitinib treatment, and adverse events during the treatment. Any treatment decision was at the physician’s discretion, based on patients’ characteristics and independent from participation to this study. After the first data entry, the follow-up information was validated with revision of clinical data, and specific queries were addressed to the participating Centre in case of inconsistent data.

In this sub-analysis, we included consecutive MF patients who received a primary treatment course with ruxolitinib of at least 14 days, discontinued the drug for at least 14 days while in chronic phase, and survived for at least 30 days after discontinuation. A total of 302 patients discontinued ruxolitinib after a median observation time of 13.9 months (range 0.5-84.5). Eighty-three patients were excluded from this analysis because they discontinued ruxolitinib in accelerated/blast phase (63) or survived less than 30 days after discontinuation (10) or discontinued ruxolitinib for less than 14 days (10). Therefore, the present analysis

comprises 219 chronic phase patients who received and stopped ruxolitinib for ≥14 days and survived for ≥30 days after discontinuation. **Figure 1** reports numbers of individuals at each stage of the study. All patients were followed until death or to data cut-off (December 1st, 2020).

Definitions

Diagnoses of primary MF (PMF) and post-polycythemia vera (PPV)/post-essential thrombocythemia (PET) MF were made according to 2016 World Health Organization criteria or International Working Group on Myelofibrosis Research and Treatment (IWG-MRT) criteria, respectively[18,19]. All patients who received treatment with ruxolitinib in the current analysis were in chronic phase (peripheral and marrow blast cells <10%). Risk category was assessed at the time patients started on ruxolitinib according to the Dynamic International Prognostic Score System (DIPSS)[20]. Histologic examination was performed at local institutions; fibrosis was graded according to the European Consensus Grading System[21]. Unfavourable karyotype was categorized as previously described[22]. Diagnosis of blast phase (BP) was made according to World Health Organization criteria, with a 20% bone marrow or peripheral blood blast threshold for diagnosis[19]. The burden of MF-related symptoms was assessed using the 10-item Myeloproliferative Neoplasm Symptom Assessment Form Total Symptom Score (MPN10-TSS)[23]. Spleen responses were assessed according to 2013 IWG-MRT/European LeukemiaNet (ELN) criteria[7].

Inadequate response included lack of spleen response (i.e. absence of spleen response with ruxolitinib therapy ≥3 months) and loss of spleen response (i.e. any increase in spleen size not meeting the initial response criteria at maximum tolerated dose)[24]. Notably, at the time patients lost a spleen response, the spleen still may have been smaller than it was at baseline.

All adverse events were defined and graded according to Common Terminology Criteria for Adverse Events (CTCAE) v 4.0. Specifically, events graded ≥2 required active systemic treatment and those graded 4 were life-threatening.

Ethical aspects

The RUX-MF study was performed in accordance with the guidelines of the Institutional Review Boards of the participating centres and the standards of the Helsinki Declaration. The promoter of this study was the Institute of Hematology "L. and A. Seràgnoli", Azienda Ospedaliera S. Orsola-Malpighi, Bologna, that obtained the approval by the Area Vasta Emilia Centro (AVEC) Ethics Commitee. The study was also approved by the local Ethics Committee of all participating Centres (protocol code – MF-2014-01) and has no commercial support.

Statistical analysis

Statistical analysis was carried out at the biostatistics laboratory of the MPN Unit at the Institute of Hematology "L. and A. Seràgnoli", Azienda Ospedaliero-Universitaria S. Orsola-Malpighi, Bologna. Continuous variables have been summarized by their median and range, and categorical variables by count and relative frequency (%) of each category. Comparisons of quantitative variables between groups of patients were carried out by Wilcoxon-Mann-Whitney rank-sum test or the Student's t-test, and association between categorical variables (2-way tables) was tested by the Fisher exact test or $\chi 2$, as appropriate. Variations in continuous and categorical variables between ruxolitinib discontinuation and rechallenge and between rechallenge and last contact on ruxolitinib were assessed using the Wilcoxon signed-rank and the McNemar test, respectively. Treatments were considered time-to-event variables, and comparisons were

carried out using Log-rank tests. Time to therapy was calculated from the first discontinuation of ruxolitinib to the start of the other therapy. Factors associated with response to ruxolitinib rechallenge were identified considering death as competing risk, according to the model of Fine and Gray, from ruxolitinib rechallenge start to the date of the response or last contact on ruxolitinib therapy. Multivariable analysis was not carried out when ≤1 covariate had a $p<0.10$ in univariate analyses. For all tested hypotheses, two-tailed p-values <0.05 were considered significant. Statistical analyses were performed using STATA Software, 15.1 (StataCorp LP, College Station TX, USA).

Results

Population on study

Overall, 219 (31.2%) out of 703 total patients were evaluable in this study. Among these 219 patients, 60 (27.4%) patients rechallenged ruxolitinib ("RUX-again" cohort), while 159 (72.6%) discontinued ruxolitinib permanently ("RUX-stop" cohort). The main demographic, clinical and hematological features at ruxolitinib start and at first/only ruxolitinib discontinuation are presented in **Table 1.** No significant differences were observed in the two cohorts. The median duration of ruxolitinib therapy before the first/only discontinuation was 16.5 (0.5-84.5) and 12.3 (0.8-79.1) months for RUX-again and RUX-stop patients, respectively (p=0.41). The median follow-up after ruxolitinib discontinuation was 18.8 months (range 1-93.7) in RUX-again and 15.5 months (range 1.3-79.8) in RUX-stop patients (p=0.21).

In the 60 RUX-again patients, the main reason for temporary discontinuation was toxicity (n=42, 70%) including grade 3-4 thrombocytopenia (38.1%), anemia (26.2%), infections (21.4%), and other adverse events comprising second primary malignancies, liver toxicity, hemorrhages and pleural effusions (14.3%). In the remaining 18 patients, ruxolitinib was discontinued due to inadequate spleen response (lack of response: 10; loss of response: 8).

Among the 159 RUX-stop patients, 75 (47.2%) discontinued ruxolitinib because of inadequate spleen response (lack of response: 56; loss of response: 19) and 15 (9.4%) stopped while in response to undergo ASCT. Adverse events caused ruxolitinib discontinuation in the remaining 69 patients (43.4%), specifically: grade 3-4 anemia (40.6%), thrombocytopenia (27.5%), infections (18.8%), and others including second primary malignancies and thromboses (13.1%). Overall, the percentage of patients who discontinued due to inadequate response was significantly higher in RUX-stop patients (p=0.004).

Efficacy of ruxolitinib rechallenge

At first ruxolitinib discontinuation, 36.2% of RUX-again patients presented with large splenomegaly (spleen palpable ≥ 10 cm below left costal margin); median TSS was 10 (TSS>20 in 31.7% of the patients). The median duration of the temporary drug discontinuation was 2 months (range 0.5-71.1) and was slightly shorter in patients who discontinued due to toxicity (median 1.3 months) than in patients who had inadequate response (median 8.2 months; p=0.05). While 38 (63.3%) patients rechallenged ruxolitinib within 3 months from first discontinuation, 12 (20%), 3 (5%) and 7 (11.7%) patients rechallenged the drug after 3-6 months, 6-12 months, and more than 12 months, respectively.

Between ruxolitinib discontinuation and rechallenge, 80% of RUX-again patients did not receive any therapy or only palliation (including corticosteroids and/or hydroxyurea and/or recombinant erythropoietin), 11.7% switched to investigational agents (including alternative *JAK2*-inhibitors, telomerase inhibitors and/or antifibrotic agents), 3.3% and 5% underwent splenectomy or ASCT. Compared to disease status at ruxolitinib stop, at rechallenge there was a significant increase of patients with larger splenomegaly and higher TSS

(**Table 2**). These variations between stop and rechallenge remained significant even when considering the categorical variables spleen ≥10 cm below costal margin (p=0.01) and TSS ≥20 (p<0.001). The ruxolitinib dose was lower at restart compared to first stop (p=0.04); however, the dose reductions were minimal since the variation in patients with a ruxolitinib dose >10 mg BID was not significant (p=0.21), with 73.3% of patients remaining in the same category at both time points. Also, no dose differences were observed between patients who discontinued due to lack/loss of spleen response and toxicity (p=0.44). Four patients were not evaluable for spleen length because they underwent splenectomy before RUX start (n. 2) or before RUX rechallenge (n. 2).

During the rechallenge period, 44.6% and 48.3% patients improved spleen and symptoms, and there was a significant increase in patients with TSS reduction (p=0.01); 12 patients (20%) continued ruxolitinib with stable/worsening spleen size but improvement in TSS. Conversely, 26.8% and 20% of patients had increase in spleen size and in symptoms, respectively.

Notably, patients who rechallenged ruxolitinib with a dose >10 mg BID had a higher probability of achieving a reduction of spleen length (SHR 2.19, 95%CI 0.99-4.86, p=0.05) and of TSS (SHR 2.67, 95%CI 1.20-5.93, p=0.02). Conversely, no association was found between spleen/TSS reduction and age ≥ 65 (p=0.81/p=0.17), male sex (p=0.34/p=0.84), hemoglobin <10 g/dl (p=0.70/p=0.62), platelet count <100x10^9/l (p=0.34/p=0.64), spleen ≥10 cm below costal margin (p=0.67/p=0.38), TSS≥20 (p=0.88/p=0.45), duration of ruxolitinib discontinuation >3 months (p=0.20/p=0.29) or >12 months (p=0.20/p=0.37), use of other therapies before rechallenge (p=0.20/p=0.37).

Overall, 31 (51.7%) patients permanently discontinued ruxolitinib because of death (32.3%), lack of response (29%), hematological toxicity (19.3%), MF progression (6.5%), infection (3.2%) or bleeding (3.2%); 6.5% of patients discontinued in good response to undergo ASCT. The percentage of RUX-again patients who permanently discontinued ruxolitinib was 20%, 33.3% and 48.3% at 6, 12 and 24 months, respectively. The median follow-up from ruxolitinib permanent discontinuation to last contact was 10.9 months (range 1.2-45). Among the 21 living patients at the time of the second discontinuation, most (54.5%) received no or palliative therapy, while 3 were treated with an investigational *JAK2*-inhibitor and 2 underwent ASCT.

Outcome of patients according to ruxolitinib rechallenge

Among the 159 RUX-stop patients, 68.5% received no therapy or only palliation including corticosteroids and/or hydroxyurea; 15.1% received ASCT, 10.7% investigational agents and 5.7% splenectomy. The use of other treatments excluding ruxolitinib was comparable in RUX-again and RUX-stop patients (investigational agents: log-rank p=0.28; ASCT: log-rank p=0.09; splenectomy, log-rank p=0.08).

From the date of first/last ruxolitinib discontinuation, a total of 25 (41.7%) RUX-again and 105 (66.0%) RUX-stop died. Causes of death were progressive MF (36.2%), infections (16.2%), second primary malignancies (8.5%), progression to BP (6.9%), and other causes (32.2%). Causes of death were comparable in RUX-again and RUX-stop patients (p=0.32).

Overall survival (OS) for the total cohort was 68.6% and 40.6% at 1 and 3 years, respectively. Notably, RUX-again patients had a significantly longer survival compared to RUX-stop patients, with a median survival of 41.1 and 23.7 months, respectively in the 2 cohorts (log-rank p=0.004) (**Figure 2**). However, OS was comparable in patients who discontinued ruxolitinib because of lack/loss of response and because of toxicity (median survival 27.9 and 27.6 months, respectively, p=0.63). Comparing patients with lack or loss of response, no survival difference was also observed (p=0.16).

Discussion

According to the 2020 National Comprehensive Cancer Network (NCCN) guidelines, possible medical alternatives beyond palliation, in case of resistance or intolerance to ruxolitinib, include use of fedratinib and investigational agents[25].

This real-world study has been completed before the availability of fedratinib in Europe and shows that ruxolitinib rechallenge was quite common after initial ruxolitinib failure, involving almost 30% of patients who discontinued the drug in chronic phase. In absence of alternative *JAK2*-inhibitors in routine practice, the rechallenge was attempted early and before other therapeutic approaches in most cases, representing an easily viable option particularly in intolerant patients.

The temporary discontinuation generally caused a significant increase in disease burden, reflecting a loss of residual control activity not only in intolerant, but also in resistant patients. Analogously, a "ruxolitinib rebound syndrome" (RDS), attributed to an acute rebound of cytokine storm soon after ruxolitinib discontinuation, has been documented in many resistant patients[26-30]. To this regard, we previously observed that thrombocytopenia (platelet count <100 x10^9/l) and large spleen (palpable ≥10 cm below costal margin) at ruxolitinib stop were significantly associated with a higher probability of RDS. The association between higher disease burden and RDS was interpreted as a non-negligible activity of *JAK2* inhibition in at least some patients with refractory MF, and may explain the observed efficacy of rechallenge also in refractory patients[26].

After the rechallenge, clinical responses were achieved by almost 50% of patients. This therapeutic efficacy is deemed to be based on a resensitization to *JAK2*-inhibition through different mechanisms including restoration of homodimeric JAK-STAT signalling[31]. However, other pathways may contribute to resistance to ruxolitinib and may be overcome with drug discontinuation[32]. The finding that only the use of high ruxolitinib doses (>10 mg BID) was associated with an increased probability of spleen and symptoms improvements corroborates the positive relationship between dose and response, shown in the COMFORT studies and in real-world evidence[2,33,34].

Notably, the lack of association between rechallenge efficacy and reasons for discontinuation probably stems from the fact that intolerance and resistance often overlap (i.e., the patient does not achieve or loses response because ruxolitinib is administered at suboptimal doses or intermittently because of intolerance). In other cases, intolerance reveals a more aggressive disease and/or an intrinsic frailty of the patient (i.e., concomitant comorbidities, polypharmacy, more frequent infections, greater thrombotic-hemorrhagic risk), resulting in reduced survival. In absence of alternative treatment strategies, ruxolitinib rechallenge may therefore be considered in virtually all patients with active disease. However, the durability of ruxolitinib rechallenge was quite short, with around 50% of RUX-again patients discontinuing permanently the drug at 2 years. This observation may suggest a strict clinical follow-up of patients during the rechallenge, and the rapid implementation of alternative therapeutic strategies when required.

Finally, survival seemed improved in RUX-again compared with RUX-stop patients, despite the use of investigational agents and ASCT was comparable in the two groups. This finding complements the survival benefit results observed in the COMFORT studies and extends real-world evidence of a survival advantage for patients treated with novel agents, including ruxolitinib rechallenge, after ruxolitinib failure[3,4].

The main constraint of this study is its retrospective nature. Indeed, suboptimal management or dosing of ruxolitinib, inadequate recognition of failure or intolerance of ruxolitinib and poor assessment of drug compliance, cannot be ruled out and may have contributed to premature drug discontinuations in the real-life. Nonetheless, the substantial number of included patients, the cooperation of hematology centres with

particular focus on MF, and the accurate revision of each case history may partially compensate these intrinsic shortcomings. We acknowledge that this limitation can hardly be avoided when dealing with a rare condition, such as MF, and a specific subpopulation, such as ruxolitinib-treated patients. On the other hand, after the approval of ruxolitinib for MF therapy, retrospective studies may represent the only and valuable source of comprehensive data and lead to personalized therapy.

Overall, these findings provide important real-life evidence that ruxolitinib rechallenge may be effective after initial ruxolitinib failure, with clinical improvements achieved in a not negligible portion of patients. However, ruxolitinib rechallenge was used mainly in intolerant patients, and was associated with a high rate of permanent drug discontinuations. The survival advantage observed in RUX-again patients highlights the role of appropriate treatment strategy and ruxolitinib use on outcome. Other *JAK2*-inhibitors and alternative drugs are currently under clinical investigation and may soon broaden the therapeutic scenario of MF further[35]. Future real-world evidence will possibly clarify whether the use of ruxolitinib rechallenge will be reduced or abandoned with the advent of new treatments in clinical practice, and what criteria should be used to select the patient to one treatment or another.

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Appendix

Center	Contributors
IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy	Emanuele Sutto Christian Di Pietro Camilla Mazzoni Federica D'Ambrosio Stefania Giaquinta
Division of Hematology and BMT, Azienda Sanitaria Universitaria Integrata di Udine, Italy	…
Division of Cellular Biotechnologies and Hematology, University Sapienza, Rome, Italy	Emilia Scalzulli Gioia Colafigli
Hematology Division, San Gerardo Hospital, ASST Monza, Italy	…
Division of Hematology, Città della Salute e della Scienza Hospital, Torino, Italy	Francesca Pirillo
Division of Hematology, Azienda Ospedaliera 'Bianchi Melacrino Morelli', Reggio Calabria, Italy	Domenica Ielo
Division of Hematology, University of Ferrara, Italy	…
Department of Hematology, Azienda USL - IRCCS di Reggio Emilia, Reggio Emilia, Italy	Katia Codeluppi
Hematology Division, Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy	Cristina Bucelli Silvia Artuso
Division of Hematology, Ospedale S. Eugenio, Rome, Italy	…
Department of Clinical Medicine and Surgery, Federico II University Medical School, Naples, Italy	…
Unit of Hematology and Clinical Immunology, University of Padova, Padova, Italy	Fabio D'Amore
Ematologia, Ospedale Businco, Università degli studi di Cagliari, Cagliari, Italy	Maria Pina Simula Olga Mulas
Section of Hematology, University of Verona, Verona, Italy	…
Unit of Blood Diseases and Stem Cell Transplantation, ASST Spedali Civili di Brescia, Brescia, Italy	…
Hematology and Stem Cell Transplant Center, AORMN Hospital, Pesaro, Italy	…
Haematology and BMT Centre, Azienda Ospedaliero-Universitaria di Parma, Parma, Italy	Monica Crugnola
Division of Hematology, AUSL di Piacenza, Piacenza, Italy	…
Hematology and Oncology, Friedrich-Schiller-University Medical Center, Jena, Germany	Carl Crodel
Hematology Unit, Ospedale Belcolle, Viterbo, Italy	…
Clinic of Hematology, Department of Internal Medicine (DiMI), IRCCS AOU San Martino-IST, Genova, Italy	Maurizio Miglino Micaela Bergamaschi
Department of Scienze Mediche, Chirurgiche e Tecnologie Avanzate "G.F. Ingrassia", University of Catania, Italy	Ernesta Lambusta

Table 1. **Patients' characteristics**. *Three RUX-stop and two RUX-again patients were splenectomized before ruxolitinib discontinuation.

Characteristics	**Study Cohort (n. 219)**	**RUX-stop (n. 159)**	**RUX-again (n. 60)**	***p value***
Age, years, median (range) at ruxolitinib start at first/only ruxolitinib stop	 67.5 (24.0-88.5) 69.3 (41.1-88.3)	 67.5 (24.0-88.5) 68.9 (24.3-91.1)	 67.4 (40.9-87.4) 69.3 (41.1-88.3)	 *0.62* *0.90*
Male sex, no. (%)	134 (64.2%)	97 (61.0%)	37 (61.7%)	*0.93*
Primary MF, no. (%)	127 (58.0%)	95 (59.8%)	32 (53.3%)	*0.39*
DIPSS risk category at ruxolitinib start, no. (%) Intermediate-1 Intermediate-2 High	 99 (45.2%) 109 (49.8%) 11 (5.0%)	 69 (43.4%) 84 (52.8%) 6 (3.8%)	 30 (50.0%) 25 (41.7%) 5 (8.3%)	 *0.30*
Hemoglobin, median (range), g/dL at ruxolitinib start at first/only ruxolitinib stop	 10.0 (6.0-16.7) 9.2 (5.0-15.9)	 9.9 (6.0-16.7) 9.1 (5.7-14.3)	 10.2 (7.0-16.4) 9.5 (5.0-15.9)	 *0.25* *0.94*
Platelet count, median (range), x 10^9/L at ruxolitinib start at first/only ruxolitinib stop	 217 (50-1400) 111 (3-870)	 202 (55-1400) 114 (3-829)	 249 (50-1026) 93 (8-870)	 *0.44* *0.27*
Leukocytes, median (range), x 10^9/L at ruxolitinib start at first/only ruxolitinib stop	 9.3 (1.1-80) 8.8 (1.3-118)	 8.9 (1.1-80) 9.1 (1.3-118)	 12.6 (2.2-78.9) 7 (1.7-81.7)	 *0.16* *0.23*
Dose, median (range), mg BID at ruxolitinib start at 3 months at first/only ruxolitinib stop	 15 (5-20) 10 (5-20) 10 (2.5-25)	 15 (5-20) 10 (5-20) 10 (5-20)	 15 (5-20) 15 (5-20) 10 (2.5-25)	 *0.21* *0.87* *0.53*
Dose>10 mg BID, no. (%) at ruxolitinib start at 3 months at first/only ruxolitinib stop	 127 (58.0%) 109 (49.8%) 86 (39.2%)	 87 (54.7%) 78 (49.1%) 64 (40.2%)	 40 (66.7%) 31 (51.7%) 22 (36.7%)	 *0.11* *0.85* *0.60*
Total Symptoms Score, median (range) at ruxolitinib start at first/only ruxolitinib stop	 20 (0-90) 10 (0-100)	 20 (0-90) 10 (0-100)	 20 (0-80) 10 (0-52)	 *0.13* *0.37*
Total Symptoms Score ≥ 20, no. (%) at ruxolitinib start at first/only ruxolitinib stop	 74 (33.8%) 67 (30.6%)	 59 (37.1%) 48 (30.2%)	 15 (25.0%) 19 (31.7%)	 *0.12* *0.86*
Spleen size BLCM, median (range), cm at ruxolitinib start at first/only ruxolitinib stop	 12 (0-38) 9 (0-40)	 11 (0-38) 9.5 (0-40)	 12 (0-29) 8 (0-28)	 *0.48* *0.56*
Spleen size ≥ 10 cm BLCM, no. (%) at ruxolitinib start at first/only ruxolitinib stop*****	 138 (63.0%) 99 (46.3%)	 101 (63.5%) 78 (50.0%)	 37 (61.7%) 21 (36.2%)	 *0.80* *0.07*
Months from MF diagnosis to ruxolitinib start, median (range)	23.6 (0-337)	22.9 (0-317)	24.0 (0.1-337)	*0.80*

Table 2. Clinical and laboratory characteristics at first stop of ruxolitinib, at rechallenge and at last contact on ruxolitinib. The doses 2.5 and 1.25 mg BID stand for 5 mg once daily and 5 mg every other day, respectively. *P-value of Wilcoxon signed-rank tests assessing the variations of variables between the first stop and rechallenge. **P-value of Wilcoxon signed-rank tests assessing the variations of variables between rechallenge and last contact on ruxolitinib. Four patients were not evaluable for spleen length because they underwent splenectomy before RUX start (n. 2) or before RUX rechallenge (n. 2).

Characteristics	**At discontinuation, median (range)**	**At rechallenge, median (range)**	**No. of pts with increased values**	**No. of pts with decreased values**	**No. of pts with stable values**	***p value****	**At last contact on ruxolitinib, median (range)**	**No. of pts with increased values**	**No. of pts with decreased values**	**No. of pts with stable values**	***p value*** **
Hemoglobin, g/dl	9.5 (5-15.9)	9.1 (6-16.2)	31	25	4	*0.22*	9.2 (5.3-15.9)	22	32	6	*0.17*
Leucocytes, $x10^9$ /l	7 (1.7-81.7)	8 (1.8-75)	33	20	7	*0.19*	*8.1 (1.3-90)*	*26*	*27*	*7*	*0.63*
Platelets, $x10^9$/l	93 (8-870)	141 (45-1305)	34	22	4	*0.01*	*114 (4-375)*	*15*	*43*	*2*	*<0.001*
Spleen length, cm below costal margin	8 (0-28)	10 (0-29)	31	8	17	*<0.001*	8 (0-34)	15	25	16	*0.12*
Total Symptoms Score	10 (0-52)	20 (0-66)	34	10	16	*<0.001*	10 (0-85)	12	29	19	*0.01*
Median ruxolitinib dose, mg BID	10 (2.5-25)	10 (2.5-25)	12	24	24	*0.04*	*10 (1.25-20)*	*18*	*10*	*32*	*0.20*

Figure 1. Study flowchart. Numbers of individuals at each stage of the study, main descriptive results and major findings are reported.

MF: myelofibrosis; RUX: ruxolitinib; TSS: Total Symptoms Score. BCM: below costal margin

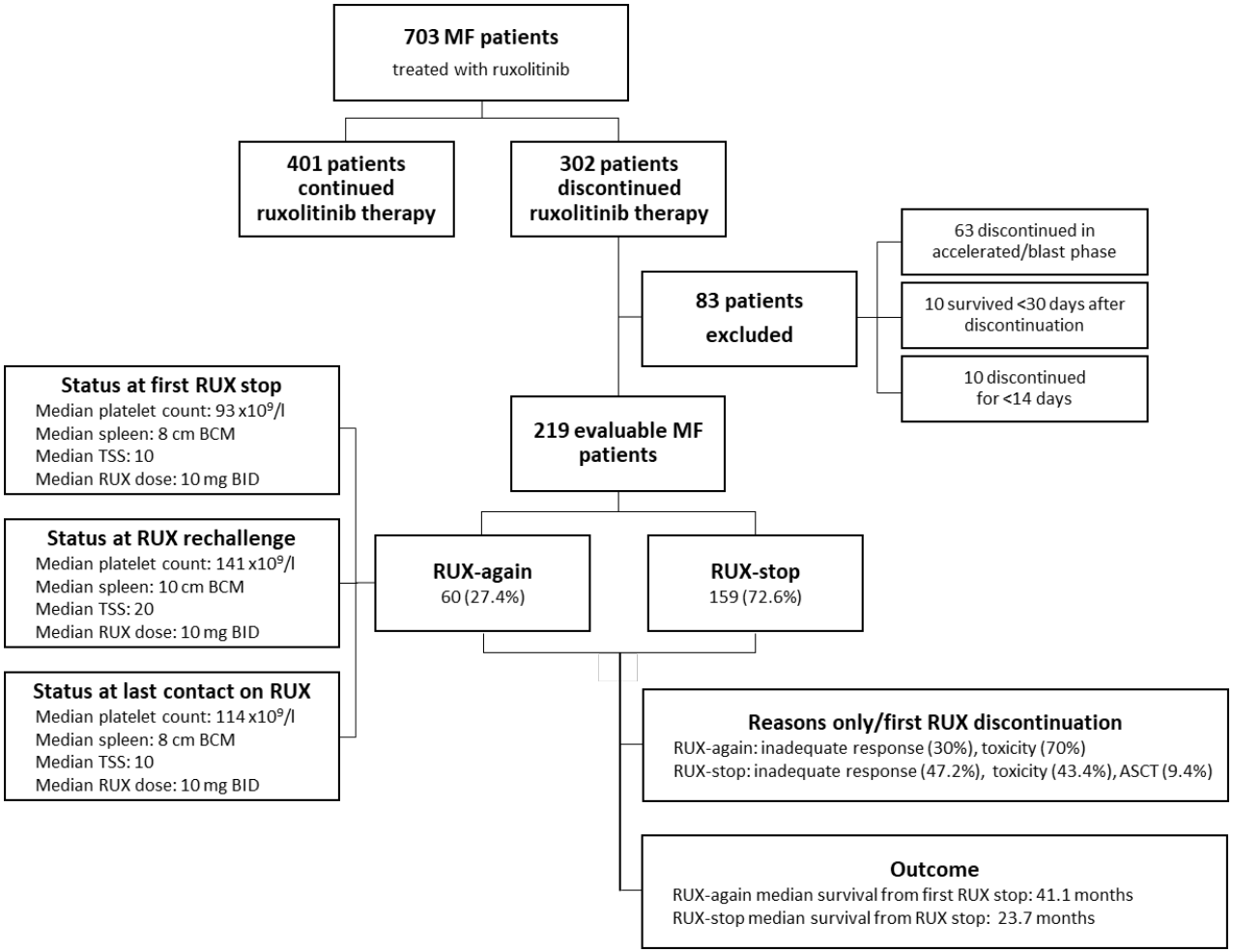

Figure 2. Overall survival according to use of ruxolitinib rechallenge. Overall survival was estimated from the date of the first/only ruxolitinib discontinuation to last contact (log-rank test).