Peripheral blasts are associated with responses to ruxolitinib and outcomes in patients with chronic-phase myelofibrosis

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BACKGROUND: The presence of peripheral blasts (PB) is a negative prognostic factor in patients with primary and secondary myelofibrosis (MF) and PB ≥4% was associated with a particularly unfavorable prognosis. Ruxolitinib (RUX) is the JAK1/2 inhibitor most used for treatment of MF-related splenomegaly and symptoms. Its role has not been assessed in correlation with PB. METHODS: In 794 chronic-phase MF patients treated with RUX, we evaluated the impact of baseline percentage of PB on response (spleen and symptoms responses) and outcome (RUX discontinuation-free, leukemia-free, and overall survival). Three subgroups were compared: PB-0 (no PB, 61.3%), PB-4 (PB 1%-4%, 33.5%), and PB-9 (PB 5%-9%, 5.2%). RESULTS: At 3 and 6 months, spleen responses were less frequently achieved by PB-4 (P = .001) and PB-9 (P = .004) compared to PB-0 patients. RUX discontinuation-free, leukemia-free, and overall survival were also worse for PB-4 and PB-9 patients (P = .001, P = .002, and P < .001, respectively). CONCLUSIONS: Personalized approaches beyond RUX monotherapy may be useful in PB-4 and particularly in PB-9 patients. Cancer 2022;0:1-6. © 2022 The Authors. Cancer published by Wiley Periodicals LLC on behalf of American Cancer Society. This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

KEYWORDS: myelofibrosis, outcome, peripheral blasts, response, ruxolitinib.

INTRODUCTION
Myelofibrosis (MF) is the most severe among the chronic Philadelphia-negative myeloproliferative neoplasms characterized by progressive splenomegaly and symptoms, cytopenia, and marrow fibrosis. Patients with chronic phase MF show a blast count below 10% both in the peripheral blood and in the bone marrow and they may have a variable survival, ranging from months to decades.1,2 The presence of peripheral blasts (PB) is a laboratory feature associated with worse outcome, ranging from months to decades.1,2 The presence of peripheral blasts (PB) is a negative prognostic factor in patients with primary and secondary myelofibrosis (MF) and PB ≥4% was associated with a particularly unfavorable prognosis. Ruxolitinib (RUX) is the JAK1/2 inhibitor most used for treatment of MF-related splenomegaly and symptoms. Its role has not been assessed in correlation with PB. METHODS: In 794 chronic-phase MF patients treated with RUX, we evaluated the impact of baseline percentage of PB on response (spleen and symptoms responses) and outcome (RUX discontinuation-free, leukemia-free, and overall survival). Three subgroups were compared: PB-0 (no PB, 61.3%), PB-4 (PB 1%-4%, 33.5%), and PB-9 (PB 5%-9%, 5.2%). RESULTS: At 3 and 6 months, spleen responses were less frequently achieved by PB-4 (P = .001) and PB-9 (P = .004) compared to PB-0 patients. RUX discontinuation-free, leukemia-free, and overall survival were also worse for PB-4 and PB-9 patients (P = .001, P = .002, and P < .001, respectively). CONCLUSIONS: Personalized approaches beyond RUX monotherapy may be useful in PB-4 and particularly in PB-9 patients. Cancer 2022;0:1-6. © 2022 The Authors. Cancer published by Wiley Periodicals LLC on behalf of American Cancer Society. This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

Recently, PB ≥4% was associated with a particularly unfavorable prognosis in a large MF cohort, including patients in accelerated (blasts, 10%-19%) and blast (blasts, ≥20%) phase. This analysis also showed that ruxolitinib (RUX), the
**Materials and Methods**

**Patients and Study Design**

After institutional review board approval, the “RUX-MF” retrospective study collected 804 chronic phase MF patients who received RUX outside clinical trials in 26 hematology centers. In 794 patients, the percentage of PB was evaluated by morphology at RUX start. Patients were grouped in 3 subcohorts: PB-0 (no peripheral blasts observed at the day of RUX start), PB-4 (peripheral blasts between 1% and 4%), and PB-9 (peripheral blasts between 5% and 9%). To increase data reliability, in PB-0 patients, the absence of blasts during evaluations before RUX start was also established.

**Definitions**

Diagnoses of PMF and post-polycythemia vera/post-essential thrombocythemia MF were made according to 2016 World Health Organization criteria or International Working Group on Myelofibrosis Research and Treatment (IWG-MRT) criteria, respectively. Patients who received treatment with RUX in the current analysis were in chronic phase (peripheral and marrow blast cells <10%). Risk category was assessed according to the dynamic prognostic score system (DIPSS). High molecular risk (HMR) mutations were evaluated by next-generation sequencing (NGS) with the myeloid panel SOPHiA Genetics (Sophia Genetics, Saint Sulpice, Switzerland) at RUX start and included *ASXL1, IDH1/2, EZH2,* and *SRSF2* according to standard definition. Leukemic transformation was diagnosed according to standard criteria. Spleen and symptom responses were assessed using IWG-MRT criteria.

**Statistical Analysis**

Comparisons of quantitative variables between groups were performed by Kruskal-Wallis and Dunn’s tests whereas association between categorical variables was tested by the *χ²* test. Variables significantly associated with RUX discontinuation/leukemic transformation/overall survival in univariate analysis (log-rank test) were considered for multivariable analyses (MVA), performed using the Cox regression model, with adjustment for delayed entry and evaluation of the model’s performance in terms of goodness of fit. For all tested hypotheses, two-tailed *P* values <.05 were considered significant. Statistical analyses were performed using STATA Software, 15.1 (StataCorp LP, College Station, Texas).

**Ethical Aspects**

The RUX-MF study was performed in accordance with the guidelines of the institutional review boards of the participating centers and the standards of the Helsinki Declaration. The promoter of this study was the L. and A. Seràgnoli Institute of Hematology (Azienda Ospedaliera S. Orsola-Malpighi, Bologna, Italy), which obtained the approval (protocol code MF-2014-01, approval date 10/06/2014, approval file number 068/2014/U) from the Area Vasta Emilia Centro Ethics Committee (Bologna, Italy; cometico@aosp.bo.it). The study was also approved by the local ethics committee of all participating centers and has no commercial support.

**Results**

**Study Cohort**

Patients were categorized according to PB at RUX start: PB-0 (no PB; *n* = 487, 61.3%), PB-4 (PB 1%-4%; *n* = 266, 33.5%), and PB-9 (PB 5%-9%; *n* = 41, 5.2%) (Table 1). DIPSS distribution was intermediate-1 (54.1%), intermediate-2 (38.3%), and high (7.6%); 47.8% had a large splenomegaly (palpable at ≥10 cm below costal margin) and 60.6% were highly symptomatic (total symptom score [TSS], ≥20). At least 1 HMR mutation was detected in 41.3% of the 167 evaluable patients (≥2 mutations in 10.8%). Among baseline characteristics, higher percentage of PB was associated with high DIPPS risk (*P* < .001), platelet count <100 × 10⁹/L (*P* < .001), fibrosis grade ≥2 (*P* < .001), and spleen length ≥10 cm (*P* = .003).

**Impact of PB on Response to RUX**

At 3 and 6 months, 26.9% and 30.4% of 672 and 619 evaluable patients achieved a spleen response, whereas 59.7% and 68.1% were in symptom response, respectively. At 3 months, spleen response (PB-0, 31.8%; PB-4, 20.6%; PB-9, 11%; *P* = .001) and symptom response (PB-0, 62.9%; PB-4, 55.5%; PB-9, 42%; *P* = .02) were less frequently achieved by PB-4 and PB-9 patients compared to PB-0 patients. This association...
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remained significant for spleen response at 6 months (PB-0, 35%; PB-4, 25%; PB-9, 13%; \( P = .004 \)) and for both spleen response (\( P = .003 \)) and symptom response (\( P = .01 \)) at any time.

**Impact of PB on Outcome**

After a median RUX exposure of 1.5 years (0.1-8.9), 491 (61.8%) patients discontinued RUX, 110 patients (13.9%) had a leukemic transformation and 365 patients (46%) died.

Median time to RUX discontinuation was 18.3 months (range, 0.37-107), and main reasons for discontinuation were hematological toxicity (17.5%), lack of spleen response (16.7%), and leukemia transformation (14.9%). Overall, the incidence rate of discontinuation and death during therapy was 48.4 per 100 patient-years. Notably, PB-0 patients discontinued less frequently because of lack/loss of spleen response (13% vs 20% at 3 years, \( P = .01 \)) than PB-9 patients. In univariate analysis, discontinuation-free survival at 2 years was 70.8% versus 59% versus 18.1% in PB-0, PB-4, and PB-9 patients, respectively (log-rank \( P < .001 \)) (Fig. 1A). In MVA, PB-4, TSS \( \geq 20 \), and \( \geq 1 \)/\( \geq 2 \) High risk mutations remained significantly associated with higher probability of RUX discontinuation (Fig. 1B).

At 2 years, leukemia-free survival (LFS) was 94% versus 88% versus 75% in PB-0, PB-4, and PB-9 patients, respectively (log-rank \( P = .004 \)) (Fig. 1C). In MVA, PB-4 and \( \geq 2 \) HMR mutations remained significantly associated with higher probability of LT (Fig. 1D).

After a median RUX exposure of 1.5 years (0.1-8.9), 365 (46%) patients died. Median overall survival (OS) was 6.4, 5.7, and 2.5 years in PB-0, PB-4, and PB-9 patients, respectively (log-rank \( P = .001 \)) (Fig. 1E). In MVA, PB-4 age \( \geq 65 \text{ years} \) and \( \geq 2 \) HMR mutations remained significantly associated with a lower survival (Fig. 1F). The use of RUX dose >10 mg twice daily at RUX start (\( P = .001 \)), at 3 months (\( P < .001 \)), and overall (\( P < .001 \)) were associated with a higher survival in univariate analysis, but not in MVA (data not shown). Causes of death included MF progression (24.7%), infection (20%), leukemic transformation (18.4%), bleeding (7.4%), second neoplasia (7.4%), heart disease (5.5%), thrombosis (3%), allogeneic stem cell transplantation (2.2%), and other unrelated causes (11.4%). Notably, PB-9 patients more frequently died due to infections (\( P = .004 \)) and leukemia transformation (\( P < .001 \)) compared to PB-0 patients.

Unfavorable association with LFS (\( P = .02 \)) and OS (\( P = .05 \)) was also confirmed in patients with HMR \( \geq 1 \).

**DISCUSSION**

Refining the prognosis of chronic phase MF patients treated with RUX and identifying the subcategories that are most at risk of therapeutic failure is of extreme relevance in the current clinical setting, which has been enriched with new therapeutic possibilities, including the recently approved JAK2-inhibitor fedratinib and other agents that are on clinical investigation for second-line use after or in combination with RUX.\textsuperscript{15,16}
Here, we analyzed whether further subclassification of PB count, an easily detectable variable, was relevant for treatment response and outcome in chronic phase patients. Overall, RUX monotherapy confirmed its efficacy in this population, with significant response rates and most patients still on therapy at 3 years. However, a higher percentage of PB was significantly associated with significantly lower response rates to RUX at 3 and 6 months. This finding is partly due to the correlation of PB with other factors that were found to be associated with worse clinical response, specifically high DIPSS risk, massive splenomegaly, and thrombocytopenia. Accordingly, patients with PB between 5% and 9% had significantly higher rates of RUX discontinuation, mainly due to lack/loss of spleen response. Moreover, where PB between 5% and 9% seem to be less impactful, the small number of patients of the PB-9 subgroup, which reduces its statistical power, must be taken into account.

The prognostic role of HMR, which was found here to be correlated with all outcome parameters, has been previously described and is certainly relevant. However, NGS evaluation is costly and sometimes unavailable. The association between higher PB percentages and higher risk of leukemic transformation and death in MVA, together with HMR mutations, confirms how much this simple laboratory finding reflects a biologically more aggressive disease and makes a strong argument for PB...
evaluation at start of RUX. Further studies might also clarify the relevance of prospective PB monitoring during RUX treatment.

The main constraint of this study is its retrospective nature. Particularly, inaccurate count of PBs, that were assessed by the treating hematologists without a centralized re-evaluation, or failure to recognize significant fluctuations in PB count before RUX start, cannot be entirely ruled out. The count of marrow blasts was not available because most patients had a dry tap and it could not be used to integrate and extend data on circulating blasts. Nonetheless, the substantial number of included patients and the use of 3 macro-subcategories, each of which included patients with a wide range of PB counts, may partially compensate these intrinsic shortcomings.

Overall, this study highlights how a lower response rate and a shorter duration of RUX benefit in PB-4, and particularly in PB-9 patients, strongly support the need for clinical trials investigating novel and/or combinatorial approaches in these patients.

FUNDING SUPPORT
This work was supported by BolognAIl (Associazione Italiana contro le Leucemie Bologna) and by Ministero della Salute Ricerca corrente.

CONFLICT OF INTEREST DISCLOSURES
Francesca Palandri received consultancy fees and/or honoraria from Novartis, Celgene, AOP, Sierra Oncology, and CTI. Massimo Breccia received honoraria from Novartis, BMS, Pfizer, and Incyte. Elisabetta Abbruzzese received honoraria from Novartis, BMS, Pfizer, and Incyte. Massimiliano Bonifacio received honoraria from Novartis, BMS, Pfizer, and Incyte. Mario Tribelli received consultancy fees and honoraria from Novartis, Malgorzata M. Trawinska received consultancy fees and honoraria from Novartis, Giuseppe A. Palumbo received honoraria and/or consultancy fees from AbbVie, AOP, AstraZeneca, BMS-Celgene, Novartis, Incyte, Janssen, and Takeda. Giulia Benevolo received honoraria from Novartis, Janssen, Agen, Takeda, and BMS. Gianni Binotto received honoraria from Novartis, Incyte, BMS-Celgene, and Pfizer. Francesco Cavadzini received honoraria from Novartis, Incyte, and Pfizer. Monica Crugnola received honoraria from Novartis and Agen. Florian H. Heidel received consultancy fees from Novartis, CTI, and Celgene and research funding from Novartis. Monica Bocchia received honoraria from Incyte, Janssen, Jazz, and Novartis. Fabrizio Pane received honoraria from Incyte, Novartis, Jazz, BMS-Celgene, AMGEN, and Gilead. Michele Cavo received consultancy fees and honoraria from Janssen, BMS-Celgene, SanoFI, GlaxoSmithKline, Takeda, Agen, OncotypeRied, AbbVie, Karyopharm, Adaptive, and Novartis. The other authors made no disclosures.

AUTHOR CONTRIBUTIONS
Francesca Palandri: Conceptualization, data curation, investigation, methodology, project administration, resources, supervision, validation, visualization, writing—original draft, and writing—review and editing. Daniela Bartoletti: Data curation, formal analysis, investigation, methodology, project administration, resources, validation, visualization, writing—original draft, and writing—review and editing. Giuseppe A. Palumbo: Conceptualization, validation, investigation, resources, and writing—review and editing. Massimo Breccia: Conceptualization, validation, investigation, resources, and writing—original draft. Alessandra Iurlo: Investigation, resources, and writing—review and editing. Massimiliano Bonifacio: Investigation, resources, and writing—review and editing. Elisabetta Abbruzzese: Investigation, resources, and writing—review and editing. Giovanni Caocei: Investigation, resources, and writing—review and editing. Elena M. Ellis: Investigation, resources, and writing—review and editing. Giuseppe Autieri: Investigation, resources, and writing—review and editing. Mario Tribelli: Investigation, resources, and writing—review and editing. Nicola Polverelli: Investigation, resources, and writing—review and editing. Maurizio Miglino: Investigation, resources, and writing—review and editing. Florian H. Heidel: Investigation, resources, and writing—review and editing. Alessia Tiegli: Investigation, resources, and writing—review and editing. Giulia Benevolo: Investigation, resources, and writing—review and editing. Eloise Beggiato: Investigation, resources, and writing—review and editing. Carmen Fava: Investigation, resources, and writing—review and editing. Francesco Cavazzini: Investigation, resources, and writing—review and editing. Novella Pugliesi: Investigation, resources, and writing—review and editing. Antonino Cuneo: Investigation, resources, and writing—review and editing. Monica Bocchia: Investigation, resources, and writing—review and editing. Costanza Bosi: Investigation, resources, and writing—review and editing. Bruno Martinotto: Investigation, resources, and writing—review and editing. Monica Crugnola: Investigation, resources, and writing—review and editing. Emanuela Ottaviani: Investigation, resources, and writing—review and editing. Giorgia Micucci: Investigation, resources, and writing—review and editing. Małgorzata M. Trawinska: Investigation, resources, and writing—review and editing. Massimo Breccia: Investigation, resources, and writing—review and editing. Daniela Cilloni: Investigation, resources, and writing—review and editing. Flavio Piano: Investigation, resources, and writing—review and editing. Rosario Sodano: Investigation, resources, and writing—review and editing. Monica Bocchia: Investigation, resources, and writing—review and editing.

REFERENCES
1. Cervantes F, Dupriez B, Passamonti F, Vannucchi AM, Morra E, Reilly JT, et al. Improving survival trends in primary myelofibrosis: an international study. J Clin Oncol. 2012;30:2981-2987.
2. Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127:2391-2405.
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;113:2895-2901.
4. Passamonti F, Cervantes F, Vannucchi AM, Morra E, Rumi E, Cazzola M, et al. Dynamic International Prognostic Scoring System (DIPSS) predicts progression to acute myeloid leukemia in primary myelofibrosis. Blood. 2010;116:2857-2858.
5. Gangat N, Caramazza D, Vaidya R, George G, Bega K, Schwager S, et al. DIPSS plus: a refined Dynamic International Prognostic Scoring System for primary myelofibrosis that incorporates prognostic information from karyotype, platelet count, and transfusion status. J Clin Oncol. 2011;29:392-397.
6. Passamonti F, Giorgiño T, Morra B, Guglielmelli P, Rumi E, Maffioli M, et al. A clinical-molecular prognostic model to predict survival in patients with post polycythemia vera and post essential thrombocytosis myelofibrosis. Leukemia. 2017;31:2726-2731.
7. Guglielmelli P, Lasho TL, Rotunno G, Mursidreddy M, Mannarelli C, Nicolis M, et al. MIPSS70: Mutation-Enhanced International Prognostic Score System for transplantation-age patients with primary myelofibrosis. J Clin Oncol. 2018;36:310-318.
8. Iurlo A, Cattaneo D, Gianelli U. Blast transformation in myeloproliferative neoplasms: risk factors, biological findings, and targeted therapeutic options. Int J Mol Sci. 2019;20:1839.
9. Paladini E, Palumbo GA, Iurlo A, Polverelli N, Benevolo G, Breccia M, et al. Differences in presenting features, outcome and prognostic
models in patients with primary myelofibrosis and post-polycythemia vera and/or post-essential thrombocytopenia myelofibrosis treated with ruxolitinib. New perspective of the MYSEC-PM in a large multicenter study. Semin Hematol. 2018;55:248-255.

10. Harrison CN, Vannucchi AM, Kiladjian JJ, Al-Ali HK, Gisslinger H, Knoops L, et al. Long-term findings from COMFORT-II, a phase 3 study of ruxolitinib vs best available therapy for myelofibrosis. Leukemia. 2017;31:775.

11. Masarova L, Bose P, Pemmaraju N, Daver NG, Zhou L, Pierce S, et al. Prognostic value of blasts in peripheral blood in myelofibrosis in the ruxolitinib era. Cancer. 2020;126:4322-4331.

12. Barosi G, Mesa RA, Thiele J, Cervantes F, Campbell PJ, Verstovsek S, et al. Proposed criteria for the diagnosis of post-polycythemia vera and post-essential thrombocythemia myelofibrosis: a consensus statement from the International Working Group for Myelofibrosis Research and Treatment. Leukemia. 2008;22:437-438.

13. Vannucchi AM, Lasho TL, Guglielmelli P, Biamonte F, Pardanani A, Pereira A, et al. Mutations and prognosis in primary myelofibrosis. Leukemia. 2013;27:1861-1869.

14. Tefferi A, Cervantes F, Mesa R, Passamonti F, Verstovsek S, Vannucchi AM, 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.

15. Mullally A, Hood J, Harrison C, Mesa R. Fedratinib in myelofibrosis. Blood Adv. 2020;4:1792-1800.

16. Venugopal S, Mascarenhas J. Current clinical investigations in myelofibrosis. Hematol/Oncol Clin N Am. 2021;35:553-575.

17. Palandri F, Palumbo GA, Bonifacio M, Tiribelli M, Benevolo G, Martino B, et al. Baseline factors associated with response to ruxolitinib: an independent study on 408 patients with myelofibrosis. Oncotarget. 2017;8:79073-79086.