RISK FACTORS FOR PROGRESSION TO BLAST PHASE AND OUTCOME IN 589 PATIENTS WITH MYELOFIBROSIS TREATED WITH RUXOLITINIB: REAL WORLD DATA

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Abstract

The impact of ruxolitinib therapy on evolution to blast phase (BP) in patients with myelofibrosis (MF) is still uncertain. In 589 MF patients treated with ruxolitinib, we investigated incidence and risk factors for BP and we described outcome according to disease characteristics and treatment strategy.

After a median follow-up from ruxolitinib start of 3 years (range 0.1-7.6), 65 (11%) patients transformed to BP during (93.8%) or after treatment. BP incidence rate was 3.7 per 100 patient-years, comparably in primary and secondary MF (PMF/SMF) but significantly lower in intermediate-1 risk patients (2.3 *versus* 5.6 per 100 patient-years in intermediate-2/high risk patients, p<0.001).

In PMF and SMF cohorts, previous interferon therapy seemed to correlate with a lower probability of BP (HR 0.13, p=0.001 and HR 0.22, p=0.02, respectively). In SMF, also platelet count $<150x10^9$/l (HR 2.4, p=0.03) and peripheral blasts ≥3% (HR 3.3, p=0.004) were significantly associated with higher risk of BP. High-risk category according to DIPSS and MYSEC-PM predicted BP in patients with PMF and SMF, respectively. Median survival after BP was 0.2 (95% CI: 0.1-0.3) years.

Therapy for BP included hypomethylating agents (12.3%), induction chemotherapy (9.2%), allogeneic transplant (6.2%), or supportive care (72.3%). Patients treated with supportive therapy had a median survival of 6 weeks, while 73% of the few transplanted patients were alive at a median follow-up of 2 years.

Progression to BP occurs in a significant fraction of ruxolitinib-treated patients and is associated with DIPSS and MYSEC-PM risk in PMF and SMF, respectively.

Introduction

Myelofibrosis (MF) is a chronic myeloproliferative neoplasm (MPN) that may arise de novo (primary MF, PMF) or post Polycythemia Vera or Essential Thrombocythemia (PPV/PET-MF, known also as secondary myelofibrosis, SMF)[1]. The median survival expectation in MF may range from less than 2 years to over 20 years according to risk category based on currently available prognostic scores (mainly, the International Prognostic Score System [IPSS] and the dynamic-IPSS [DIPSS] for PMF or the Myelofibrosis Secondary to PV and ET Collaboration Prognostic Model [MYSEC-PM] for SMF)[2-4]. Recently, also molecular- and cytogenetic-based models for PMF have been proposed to refine prognostication in transplant-age patients[5,6]. Main causes of death include progression of MF, thrombotic/haemorrhagic events, second primary malignancies and infections[7]. Also, blast phase (BP) is the terminal and most incurable phase of all MPNs[8]. From diagnosis of BP, outcome is extremely poor, with a predicted median survival shorter than 5 months[9-11].

To-date, several retrospective observational studies have investigated epidemiology and risk factors for BP-MPNs, including presence of circulating blasts (≥3%), thrombocytopenia (platelet count <100x10^9/L), unfavourable cytogenetic/molecular alterations and high IPSS risk category[12,13]. In a cohort of 805 SMF patients, blasts ≥ 3%, non-*CALR* genotype and longer duration of PV/ET were found to correlate with an increased risk of BP, and the MYSEC-PM predicted BP evolution[14]. Very recently, a predictive model for BP evolution has been proposed in patients with PMF, that includes presence of *IDH1*, *SRSF2, or ASXL1* mutations, circulating blasts ≥3%, age >70 years, and presence of moderate or severe anemia[15]. However, most results have been generated in cohorts of ruxolitinib-naïve patients and little data are available for patients with SMF. Ruxolitinib (Jakavi/Jakafi, Novartis/Incyte) is a selective *JAK1/JAK2* inhibitor that proved its superiority in reducing splenomegaly and symptoms over placebo and best available therapy in the two prospective randomized controlled COMFORT trials[16-18]. While ruxolitinib has been associated with reduced cachexia and possible survival advantage, a reduction in overall number or timing of BP transformation has never been demonstrated[16].

Ruxolitinib now represents the standard of care for patients with intermediate to high-risk symptomatic MF and is therefore administered to most patients who subsequently progress to BP. The objective of this study is to assess real-world data on (i) incidence of BP during ruxolitinib therapy, and its clinical/laboratory correlates, (ii) treatment strategy at BP evolution, and (iii) outcome and predictors for survival in BP patients previously treated with ruxolitinib.

Methods

A multicentre observational retrospective study on patients with MF requiring ruxolitinib was conducted in 20 European Hematology Centres as previously described[19]. Data were extracted from an electronic database that included consecutive patients with chronic-phase MF treated with

ruxolitinib from June 2011 in participating Centres. The promoter of the study was the Institute of Hematology "L. and A. Seràgnoli", Sant'Orsola-Malpighi University Hospital, in Bologna. This coordinating Centre performed the analyses. Data were retrieved by the hematologists of all participating Centres. The study has no commercial support. All patients were followed until death or to data cut-off (June 2019). Risk category was assessed at ruxolitinib start according to the DIPSS or the MYSEC-PM in patients with PMF or SMF, respectively[4]. Diagnosis of BP was made according to WHO criteria, with a 20% marrow or peripheral blood blast threshold for diagnosis with major response categories being complete remission (CR) and complete remission with incomplete blood recovery (CRi)[20,21]. Spleen and symtpoms responses to ruxolitinib were assessed according to 2013 IWG-MRT/ELN criteria[22]. Reticulin staining was scored by experienced pathologists using a scale of 0–3 on the base of the EUMNET consensus[23]. Chromosomal abnormalities were considered clonal if the same structural abnormality or extra chromosome appears in at least two and monosomy in at least three metaphases[24]. Cytogenetic alterations were categorized as unfavorable according to the DIPSS-plus classification (+8, −7/7q−, i(17q), −5/5q−, 12p−, inv(3), and 11q23 abnormalities and complex karyotype defined as the presence of three or more distinct structural or numeric abnormalities)[25]. Anemia and thrombocytopenia were classified as related to BP evolution if BP occurred within 3 months of the onset of anemia/thrombocytopenia, which were not reversible with ruxolitinib withdrawal. At the time of BP evolution, cytogenetic and molecular studies were performed at discretion of the treating hematologist, mainly based on the patient's general health status and subsequent practical relevance of cytogenetic/molecular testing. DNA was extracted from peripheral blood samples for targeted next-generation sequencing (NGS) or by Sanger sequencing. NGS was performed by TruSight Myeloid Sequencing Panel (Illumina; San Diego, CA) on the MiSeq benchtop genome sequencer (Illumina) as already described[26]. Since the patients were progressed to acute myeloid leukemia (AML), only mutations that define molecular risk in AML were investigated, specifically: *ASXL1, RUNX1, TP53, FLT3*-internal tandem duplication(ITD)*, NPM1, IDH1/2*[27]. The study was approved by the IRB of each Institution and was conducted according to the Helsinki declaration.

Statistical analysis

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 and association between categorical variables was tested by the $\chi 2$ test. McNemar's test was employed to assess whether a statistically significant change in proportions occurred on a dichotomous trait (unfavourable karyotype) at two time points (ruxolitinib start and BP evolution) in the same population. Risk factors were identified conducting a time-to-event (BP) analysis using

the Fine & Gray model with death/allogeneic stem cell transplant (alloSCT) as competing risks. Variables tested for association with BP were: age ≥65 years, male sex, transfusion-dependency, platelet <150x10^9/l, peripheral blasts ≥3%, marrow fibrosis grade, *CALR*-unmutated genotype, unfavourable karyotype, spleen length (≥10 cm), MPN-10 total symptoms score (TSS) (≥20), previous splenectomy, hydroxyurea (HU), alkylating agents (busulfan or pipobroman), and interferon (IFN) use, time from MF diagnosis to ruxolitinib start, previous PV *versus* ET and PV/ET duration. The thresholds of platelet and blasts were used in analogy to the MYSEC analysis[14]. Twenty was the median TSS value and had already showed to correlate with response to ruxolitinib in a previous analysis from our group[19].

Supportive therapy included: red blood cells/platelet transfusions, corticosteroids, and recombinant erythropoietin. Differences in Cumulative Incidence Functions among risk categories of each score were also calculated in order to explore whether DIPSS and MYSEC-PM categories were associated with BP occurrence. Overall survival (OS) was defined as the time between BP diagnosis and patient death.

Results

Study cohort

At data cut-off in June 2019, 589 MF patients were included in the dataset and observed for 1833 patient-years from ruxolitinib start (median [range]: 2.95 [0.1-7.7] years). Baseline characteristics of the patient population are reported in Table 1. Diagnosis was PMF in 304 pts (51.6%), PPV-MF in 164 pts (27.8%) or PET-MF in 121 (20.6%); 58.4% patients were males. Molecular status was evaluable in 530 patients, specifically: *JAK2*^{V617F} (n. 437, 82.5%), *CALR* mutations (n. 60, 11.3%) and *MPL*$^{W515K/L}$ (n. 6, 1.1%); 27 (5.1%) were triple negatives.

Median time from MF diagnosis to ruxolitinib initiation was 1.3 years (range 0.1-32.8). Before ruxolitinib therapy, 297 patients (50.4%) received hydroxyurea (HU) alone, 37 patients (6.3%) were treated with alkylating agents (ALK), alone or in combination, and 29 patients (5%) were treated with interferon-alpha (IFN) (alone or in sequential therapy with anagrelide and/or HU). Median IFN therapy duration was 1.8 years (range 0.05-11.6), while median time from IFN discontinuation to ruxolitinib start was 7.4 years (range 0.01-23.5). Notably, both IFN and anagrelide were administered to patients with ET or pre-fibrotic PMF. Immunomodulating agents (IMiDs) (thalidomide/pomalidomide) and investigational *JAK2*-inhibitors were administered in 1.5% and 1.9% of the patients, respectively. The remaining 206 (34.9%) have received no or supportive therapy only.

At ruxolitinib start, the median age was 68 years (range 24-88). DIPSS distribution in PMF was: intermediate-1 (47.8%), intermediate-2 (45.7%), and high (6.5%). According to DIPSS and MYSEC-

PM, patients with SMF were categorized at low (0% / 11.2%), intermediate-1 (58.6% / 43.1%), intermediate-2 (33.7% / 31.2%) and high (7.7% / 14.5%) risk, respectively.

A total of 161 (27.3%) patients were transfusion-dependent and 26 out of 377 (6.9%) evaluable patients carried an unfavourable karyotype. Overall, 199 patients (33.7%) had a grade 3 marrow fibrosis (PMF: 35.2%; PPV/PET-MF: 32.1%). Patients with PMF and SMF showed similar clinical features, except for baseline levels of hemoglobin and platelets, which were significantly higher in patients with SMF (p<0.001). Median observation time from ruxolitinib start to last contact was 3 years (0.1-7.7). During ruxolitinib therapy, 141 (28%) and 354 (69%) of evaluable patients achieved a spleen or a symptom response at 3 months, respectively.

Incidence of blast phase evolution and clinical-molecular correlates

Overall, 65 (11%) developed myeloid BP. No lymphoid BP was observed. Median time from MF diagnosis to BP evolution was 3.5 years (range 0.2-18.9), comparable in PMF and SMF. Median age at BP evolution was 71 years (range 44-89). BP was the cause of ruxolitinib withdrawal in 61 patients after a median drug exposure of 1.2 years (range 0.1-6.8). Four patients progressed to BP after a median time from ruxolitinib discontinuation of 2.4 years (range 2.2-3.3). In 5 cases, BP evolution followed splenectomy. Blast phase incidence rate was 3.7 per 100 patient-years of follow-up (95% CI: 2.9-4.7 per 100 patient-years). The cumulative incidence of BP accounting for death as competing risk was 14.5% and was comparable in PMF *versus* SMF (p=0.23) and in PPV-MF *versus* PET-MF (p=0.71) **(Figure 1)**.

At the time of BP diagnosis, most patients presented with anemia and thrombocytopenia (median [range] haemoglobin: 8.8 [6.3-14.8] g/dl, median [range] platelet count: 50 [4-649] x 10^9/l); peripheral blasts were ≥30% in 40% of the patients. Karyotype was evaluable in 23 (35.4%) BP patients and resulted unfavourable in 13 (56.5%) patients. Overall, a significant increase of unfavourable karyotypes from the start of ruxolitinib therapy to BP was observed. Indeed, 50% of patients that had a normal karyotype at ruxolitinib start presented an unfavourable karyotype at BP evolution (McNemar test, p=0.01). At the time of BP, mutation associated with high-risk AML were evaluated in 14 patients. High risk mutations were detected in 5 patients, specifically: *IDH1* (1 patient), *TP53* (2 patients), and *FLT3*-internal tandem duplication (2 patients).

Baseline predictors of blast phase evolution

The probability of BP evolution in PMF was significantly reduced by previous IFN use (p<0.001) (**Figure 2a**). In SMF, predictors for BP in univariate analysis were platelet <150 x10^9/l (p=0.001), blasts ≥3% (p=0.002), grade 3 marrow fibrosis (p=0.03) and PV/ET duration ≥ 10 years (p=0.02); conversely, previous IFN use significantly reduced the risk of BP (p=0.02). In multivariable analysis,

platelet <150 x10^9/l (HR 2.4, 95% CI 1.1-5.4, p=0.03), blasts ≥3% (HR 3.3, 95% CI 1.4-7.5, p=0.004) and previous IFN (HR 0.1, 95% CI 0.02-0.8, p=0.04) remained significant in SMF (**Figure 2b**).

Overall, 287 intermediate-1 risk patients according to DIPSS or MYSEC-PM were treated with ruxolitinib. The incidence rate of BP was 2.3 per 100 pt-yrs in intermediate-1 patients compared to 5.6 per 100 pt-yrs in intermediate-2/high risk patients (p<0.001). Patients at intermediate-1 risk more frequently had a starting and cumulative ruxolitinib dose >10 mg BID (67.8% *versus* 57.2%, p=0.015 and 60.2% *versus* 50.9%, p=0.035, respectively), and had a significantly longer ruxolitinib exposure (2.8 *versus* 1.6 years, p<0.001).

High DIPSS risk significantly predicted BP in PMF (p=0.04, HR [95% CI]: 2.6 [1.1-6.5]) but not in SMF (p=0.40) (**Figure 3a and 3b**). In this latter cohort, only the MYSEC-PM was associated with BP (p=0.02, HR 1.7 [95% CI]: [1.1-2.8]) (**Figure 3c**). Estimated HRs, in reference to the lower score category, were: 1.10 for intermediate-1, 1.82 for intermediate-2, and 4.04 for high risk. HR for high risk patients, comparing to all lower risk groups, was 3.53 (95% CI: 1.53-8.11). Notably, IFN use was independently associated with a lower risk of BP evolution in PMF and SMF, without being significantly associated to lower DIPSS and MYSEC-PM risk categories (p=0.11 and p=0.36, respectively).

Treatment strategies and outcome after blast phase evolution

Most patients (72.3%) only received supportive therapy because of poor performance/health status or active infection. Ten patients (15.4%) received AML-like induction chemotherapy, followed by alloSCT in 4 cases (2 patients in CR); 8 patients (12.3%) received hypomethylating agents (HMA).

Overall, 54 (81.8%) BP patients died, with a median survival of 0.2 years (95% CI: 0.1-0.3). Survival was significantly better for transplanted patients, with 73% alive at a median follow-up of 2 years, regardless of response to previous induction therapy. Median survival of patients who received HMA/chemotherapy without transplant was not significantly better than patients who received supportive care (0.4/0.3 versus 0.1 years, p=0.51) **(Figure 4).**

Discussion

The present study represents the first data collection of patients progressing to BP under ruxolitinib treatment. We observed that the incidence of BP was overall comparable to that reported in previous cohorts of ruxolitinib-naïve patients[7], and that shorter time from MF diagnosis to ruxolitinib start did not significantly reduce the probability of subsequent BP. Taken together, these observations may indirectly suggest that ruxolitinib does not significantly modify the

probability of BP transformation or delay its occurrence. However, we acknowledge that a definitive conclusion would require a matched control cohort of patients not treated with ruxolitinib.

In PMF, the only predictor of BP evolution was the DIPSS category, which has already been associated with the risk of BP in a cohort of ruxolitinib-naive patients, with higher-risk patients having a 7.8-fold and 24.9-fold higher risk of developing BP compared to those in the low-risk category[28]. Notably, higher DIPSS categories include patients with lower hemoglobin, increased peripheral blasts count, and older age. Taken altogether, these features are similar to those already reported in previous retrospective cohorts, which identified the presence of circulating blasts ≥3%, age >70 years and anemia, together with high-molecular risk mutations, as major risk factors for BP[15]. In SMF, the main predictors of progression were reduced platelet count (<150x10^9/l) and increased circulating blasts (>3%) at ruxolitinib start. Both parameters reflect a greater disease severity and are included among risk factors for inferior survival in the MYSEC-PM score, which is dedicated to SMF[2]. Recently, we have shown that MYSEC-PM, and not DIPSS, may predict the outcome of patients with SMF treated with ruxolitinib[29]. Here, we demonstrate that MYSEC-PM is also able to predict BP in ruxolitinib-treated patients. Overall, these data confirm that PMF and SMF are two distinct diseases in which also prognostic factors need to be differentiated[30].

Notably, in both PMF and SMF patients a previous use of IFN was associated with reduced evolution to BP regardless of DIPSS/MYSEC-PM risk category. This result may support a possible disease-modifying action of IFN in MF[31]. However, it is important to acknowledge that IFN was reserved to patients with earlier diseases (i.e.: pre-fibrotic PMF) that are also at lower risk of BP evolution. Finally, this study encapsulates only 29 patients treated with IFN across both cohorts, therefore the numbers are rather small to make significant conclusions.

Conversely, no significant impact of previous exposure to DNA-damaging agents on incidence of BP was detected, confirming the hypothesis that MF biology, rather than previous therapies, may affect prognosis. Accordingly, a large international nested case-control study including 1881 MPN patients did not show excess risk of carcinoma and hematological second cancer in patients treated with pipobroman or combination therapies compared with unexposed patients[32]. However, increased incidence of BP was shown in other studies when exposed to pipobroman[33-35].

The current study confirms the poor survival of BP previously reported in ruxolitinib-naïve patients[10,11,36,37]. The role of clonal evolution is here difficult to establish. Indeed, only a minority of patients underwent cytogenetic and molecular analyses before ruxolitinib start, in accordance with international recommendations that do not require prospective monitoring in MF[38]. Also, clonal evolution at the time of BP progression was studied only in the few patients with a good performance status. Overall, there are no data to support that the clonal evolution observed in some cases was due to ruxolitinib therapy rather than to the natural course of the disease[11,39-41].

The analysis of treatment outcomes confirmed previous experiences on the use of AML-like induction chemotherapy and demethylating agents[42,43]. Also, we confirmed that all long-term (> 1 year) survivors had undergone transplantation. Indeed, the median survival of patients receiving HMA or chemotherapy without transplantation was not significantly longer than that of patients receiving only supportive care. Nonetheless, a trend for superior survival (16 *versus* 6 weeks) was observed in the first group of patients (probably due to better performance status and therapeutic efficacy) and may be clinically significant.

Overall, the present study suggests that BP evolution is intrinsically associated with the nature of MF and seems to be neither promoted, nor prevented, by ruxolitinib. From a practical point of view, the association of DIPSS/MYSEC-PM risk with BP evolution validates the use of these scores in MF patients at the start of ruxolitinib therapy and reinforces the recommendation for close hematological monitoring in high-risk categories. Finally, since long-term responses could be achieved only with allogeneic transplantation, this study confirm that transplant eligibility should be evaluated, without delay, in all high-risk fit patients, even in case of a good response to ruxolitinib[44].

Conflict of Interest Statement

F.P., M.T. and M.M.T.: consultancy and honoraria from Novartis. M.Br., M.Bo., A.Iu., E.A., and N.S.: honoraria from Novartis, Incyte, Pfize, BMS. G.B.: honoraria from Novartis, Janssen, Amgen. R.L.: honoraria from Janssen. F.H.: consultancy for Novartis, Celgene, CTI, and Research Funding from Novartis. F.C.: honoraria from Novartis, Incyte, Pfize. M.Cr.: honoraria from Novartis, Celgene, Janssen, BMS. A.Is.: honoraria from Novartis, Gilead, Janssen. M.K.: membership on advisory committees for Novartis, Janssen. G.S.: honoraria from Abbvie, Roche, Takeda. R.M.L.: honoraria from Gilead, Novartis, Sanofi, Milteny. M.Ca.: membership on advisory committees and honoraria from Celgene, Janssen, BMS, Sanofi, Novartis, Takeda, Amgen. G.A.P.: honoraria from Novartis, Celgene, Janssen, Amgen, Hospira, Teva.

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Table 1. Patients characteristics at ruxolitinib start. PMF: primary myelofibrosis. SMF: secondary myelofibrosis. PPV-MF. Post polycythemia vera myelofibrosis; PET-MF: post essential thrombocythemia myelofibrosis. DIPSS: dynamic International prognostic Score System[4]. MYSEC-PM: Myelofibrosis Secondary to PV and ET Collaboration Prognostic Model[2]

Characteristics	Median (range) / n. (%)
Age, years	68 (24-88)
Male sex	344 (58.4%)
PMF	304 (51.6%)
SMF	285 (48.4%)
PPV-MF	164 (27.8%)
PET-MF	121 (20.6%)
Mutational status, on 530 evaluable patients	
***JAK2*^{V617F}**	437 (82.5%)
CALR	60 (11.3%)
MPL	6 (1.1%)
Triple negative	27 (5.1%)
Hemoglobin, g/dl	10.7 (5.7-17.9)
Leucocytes, x10^9/l	11.3 (1.3-155)
Circulating blasts, %	0 (0-9)
Platelets, x10^9/l	256 (50-1887)
DIPSS category, on 304 PMF patients	145 (47.8%)139 (45.7%)20 (6.5%)
Intermediate-1	
Intermediate-2	
High	
MYSEC-PM category, on 285 SMF patients	
Low	32 (11.2%)
Intermediate-1	123 (43.1%)
Intermediate-2	89 (31.2%)
High	41 (14.5%)
Grade 3 marrow fibrosis	199 (33.7%)
Large splenomegaly (palpable ≥10 cm below left costal margin)	367 (62.3%)
Total Symptoms Score (TSS)	20 (0-100)
Time from MF diagnosis to ruxolitinib start, years	1.3 (0-32.8)

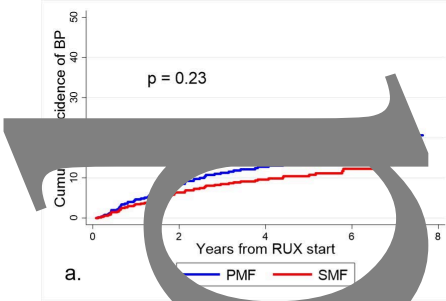
Figure Legends

Figure 1. Cumulative incidence of blast phase (BP) accounting for death and stem cell transplant as competing risks, according to the diagnosis of primary (PMF) or secondary (SMF) myelofibrosis (a) and to the diagnosis of myelofibrosis post-Polycythemia Vera/Essential Thrombocythemia (PPV/PET-MF) (b).

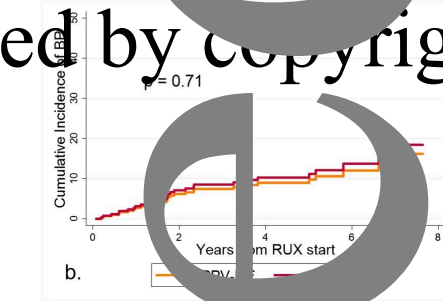
Figure 2. Univariate competing risks analysis of baseline risk factors predictive for blast phase transformation in patients with PMF (a) and SMF (b).
Karyotype was unfavourable in 27 (7.1%) out of 381 evaluable patients: 14 (7.3%) PMF and 13 (7.1%) SMF. Unfavourable alterations in PMF/SMF were, specifically: trisomy 8 (42.9%/23.1%), complex (21.4%/30.7%), del7 (21.4%/7.7%), del5 (14.3%/7.7%), inv3 (0%/15.4%) and 11q23 rearrangement (0%/15.4%). Variables with p-value < 0.20 in univariate analysis were considered for multivariable analysis and collinearity amongst variables was detected by means of Pearson correlation test. Considering collinearity, DIPSS and MYSEC were voluntarily excluded from the multivariable analyses because the factors which build these scoring systems are singularly evaluated in this analysis. TSS: Total Symptoms score. HR: Hazard Ratio. CI: Confidence Interval. HU: Hydroxyurea. IFN: Interferon.

Figure 3. Cumulative Incidence of blast phase (BP) transformation according to DIPSS risk score in PMF (a), DIPSS risk score SMF (b) and MYSEC-PM risk score in SMF (c).

Figure 4. Overall survival according to treatment strategy after blast phase evolution.

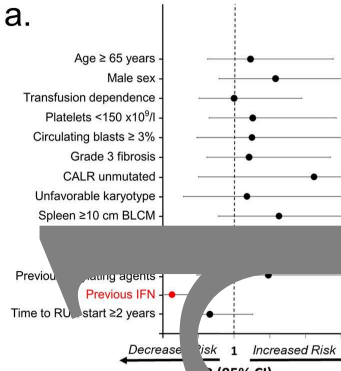


a.



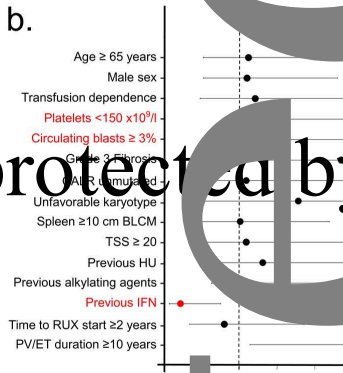
b.

a.



	UNIVARIATE HR (95% CI)	p	MULTIVARIABLE HR (95% CI)	p
Age ≥ 65 years	1.23 (0.63-2.39)	0.54		
Male sex	1.58 (0.79-3.18)	0.19	1.48 (0.74-2.97)	0.27
Transfusion dependence	1.00 (0.51-1.95)	1.00		
Platelets <150 x10^9/l	1.26 (0.65-2.43)	0.49		
Circulating blasts ≥ 3%	1.25 (0.48-3.25)	0.65		
Grade 3 fibrosis	1.21 (0.62-2.35)	0.57		
CALR unmutated	2.12 (0.50-9.04)	0.31		
Unfavorable karyotype	1.18 (0.29-4.77)	0.82		
Spleen ≥10 cm BLCM	1.63 (0.78-3.36)	0.19	1.56 (0.75-3.23)	0.23
	1.22 (0.62-2.42)	0.56		
	1.37 (0.73-2.59)	0.33		
Previou ating agents	1.48 (0.48-4.54)	0.49		
Previous IFN	**0.13 (0.03-0.37)**	**0.001**	**0.09 (0.02-0.24)**	**<0.001**
Time to RU start ≥2 years	0.66 (0.35-1.26)	0.21		

b.



	UNIVARIATE HR (95% CI)	p	MULTIVARIABLE HR (95% CI)	p
Age ≥ 65 years	1.12 (0.52-2.41)	0.77		
Male sex	1.10 (0.52-2.33)	0.80		
Transfusion dependence	1.21 (0.48-3.05)	0.68		
Platelets <150 x10^9/l	**3.57 (1.64-7.75)**	**0.001**	**2.4 (1.05-5.43)**	**0.03**
Circulating blasts ≥ 3%	**4.00 (1.68-9.53)**	**0.002**	**3.3 (1.45-7.51)**	**0.004**
Grade 3 Fibrosis	**2.35 (1.05-5.07)**	**0.03**	1.83 (0.85-4.19)	0.12
CALR unmutated	1.07 (0.5-4.72)	0.91		
Unfavorable karyotype	1.78 (0.41-7.72)	0.44		
Spleen ≥10 cm BLCM	1.01 (0.46-2.19)	0.99		
TSS ≥ 20	1.09 (0.47-2.55)	0.83		
Previous HU	1.31 (0.58-2.97)	0.52		
Previous alkylating agents	1.60 (0.63-4.07)	0.32		
Previous IFN	**0.22 (0.07-0.75)**	**0.02**	**0.14 (0.02-0.86)**	**0.04**
Time to RUX start ≥2 years	0.80 (0.34-1.86)	0.61		
PV/ET duration ≥10 years	**2.71 (1.14-6.42)**	**0.02**	2.17 (0.90-5.24)	0.09