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Differences in presenting features, outcome and prognostic models in patients with Primary Myelofibrosis and post Polycythemia vera/post Essential Thrombocythemia Myelofibrosis treated with ruxolitinib.
New perspective of the MYSEC-PM in a large multicenter study

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Differences in presenting features, outcome and prognostic models in patients with Primary Myelofibrosis and post Polycythemia vera/post Essential Thrombocythemia Myelofibrosis treated with ruxolitinib. New perspective of the MYSEC-PM in a large multicenter study

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ABSTRACT

Recently, the Myelofibrosis Secondary to PV and ET Prognostic Model (MYSEC-PM) was introduced to assess prognosis in myelofibrosis (MF) secondary to Polycythemia Vera and Essential Thrombocythemia (post-PV/post-ET MF), replacing the IPSS/DIPSS that was applied for primary MF (PMF). In a cohort of 421 ruxolitinib-treated patients (post-PV/post-ET MF: 44.2%), we evaluated: 1) disease phenotype, responses and toxicity to ruxolitinib; 2) performance of the MYSEC-PM in post-PV/post-ET MF. While the IPSS failed to correctly stratify post-PV/post-ET MF patients at diagnosis, the MYSEC-PM identified four risk categories projected at significantly different survival probability ($p < 0.001$). Additionally, the MYSEC-PM maintained a prognostic value in post-PV/post-ET MF also when used over time, at ruxolitinib start. Notably, the MYSEC-PM reclassified 41.8% and 13.6% of patients into a lower and higher-risk category, respectively. Finally, patients at intermediate-1 risk had significantly higher spleen responses and lower hematological toxicities compared to higher-risk patients. Compared to PMF, post-PV/post-ET MF presented a more hyper-proliferative disease, with higher leukocyte/platelet count and hemoglobin levels both at diagnosis and at ruxolitinib start. Despite comparable response rates, post-PV/post-ET MF had lower rate of ruxolitinib-induced anemia and thrombocytopenia at 3 and 6 months. The study validates MYSEC-PM in post-PV/post-ET MF prognostication. Post-PV/post-ET MF represents a separate entity compared to PMF in terms of clinical manifestations and toxicity to ruxolitinib.

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INTRODUCTION

Myelofibrosis (MF) is a rare chronic hematological neoplasia characterized by progressive splenomegaly, invalidating systemic symptoms (i.e.: fatigue, night sweats, weight loss, fever) and cytopenia, along with bone marrow fibrosis and increased risk of evolution into acute leukemia and early death.^{1, 2} MF pathogenesis is mainly based on the hyperactivation of the JAK-STAT pathway, causing uncontrolled myeloproliferation and abnormal release of pro-inflammatory cytokines. Most patients with MF carry mutations in three “driver” genes (*JAK2*, in 60% of the cases; *CALR*, 20%; *MPL*, 10%) with around 10% of “triple-negative” cases.^{3, 4} Additionally, “subclonal” mutations in five genes (*ASXL1*, *IDH1/2*, *EZH2*, *SRSF2*) were found to be associated with worse prognosis.^{5, 6}

MF may present as primary disease (PMF) or secondary to Polycythemia Vera or Essential Thrombocythemia, more specifically referred to as post-PV and post-ET MF.^{7, 8} Patients with post-PV/post-ET MF may exhibit a substantially different disease compared to patients with PMF in terms of clinical presentation, cytogenetic abnormalities, molecular background and prognosis.⁹ In a retrospective study of 359 patients with post-PV/post-ET MF, the *JAK2*^{V617F} and *CALR* allele burden was significantly higher in post-PV MF and/or post-ET MF. While allele burden and type of driver mutation did not correlate with overall survival (OS), triple negativity was associated with significant reduction of OS in post-ET MF, similarly to PMF. Among high risk mutations, only *SRSF2* mutations were associated with reduced survival, and only in post-ET MF. In a larger study including 685 patients with post-PV/post-ET MF, OS was significantly better in *CALR*-mutated patients, with no distinctions across the type of *CALR* mutations. Also, blast phase was more frequent in *JAK2*^{V617F}-mutated post-ET MF and in triple negative post-PV/post-ET MF compared to *CALR*-positive patients.¹⁰⁻¹³

The standard risk assessment in MF is currently based on three risk scores: the International Prognostic Scoring System (IPSS) at diagnosis, and the dynamic-IPSS (DIPSS) /DIPSS-plus during disease course. All these risk scores evaluate five clinical/laboratory parameters (specifically, age >65 yr, leukocyte >25x10⁹/l, circulating blast cells ≥1%, hemoglobin <10 g/dl and presence of either one of the three constitutional symptoms- night sweats, weight loss and fever). The DIPSS-plus requires also the availability of cytogenetic data, together with the need for red blood cell transfusion and the platelet count. Despite IPSS, DIPSS and DIPSS-plus were created and validated in PMF patients, they have been widely used also in post-PV/post-ET MF patients in routine clinical practice.¹⁴⁻¹⁶

Very recently, the Myelofibrosis Secondary to PV and ET Prognostic Model (MYSEC-PM) was developed to specifically predict survival in post-PV/post-ET MF in a large international cohort and validated in an independent study from the Spanish registry.¹⁷ This new score includes six clinical/molecular characteristics, specifically: hemoglobin (<11 g/dl, 2 points), circulating blasts (≥3%, 2 points), genotype (*CALR*-unmutated, 2 points), platelet count (<150x10⁹/l, 1 point), constitutional symptoms (1 point) and age (0.15 points to any year of age). The MYSEC-PM may stratify patients at diagnosis into four risk categories with different survival: low (median OS: not reached), intermediate-1 (9.3 yrs), intermediate-2 (4.4 yrs) and high (2 yrs).¹¹

However, the MYSEC-PM was retrospectively applied to patients who very likely received different treatments after post-PV/post-ET MF diagnosis. The extreme heterogeneity of the therapeutic strategies over the time could possibly have had an impact on life expectancy of these patients. Particularly, ruxolitinib (RUX) is the first and only commercially available *JAK1/2* inhibitor that is indicated for the treatment of MF-related splenomegaly and symptoms.^{18, 19} Despite methodological caveats, in both Controlled Myelofibrosis Study with Oral JAK Inhibitor Treatment (COMFORT-I/II) registrative trials, and in a historical comparison, ruxolitinib therapy resulted also in a survival advantage.^{20, 21, 22}

Here, we report the outcome of a large cohort of MF patients homogeneously treated with ruxolitinib, with the aims to: 1) validate the MYSEC-PM score in the cohort of homogeneously treated post-PV/post-ET MF patients; 2) evaluate the impact of risk categories on responses and toxicity; 3) evaluate the differences between PMF and post-PV/post-ET MF and also between post-PV MF and post-ET MF in terms of clinical presentation, response to ruxolitinib therapy, toxicity and survival.

PATIENTS AND METHODS

Patients and treatments

An electronic database was established to collect clinical, molecular and laboratory data on MF patients treated with ruxolitinib in 23 European Hematology Centers as previously described.²³ Between June 2011 and November 2016, data on 462 consecutive MF patients were retrospectively collected. A total of 421 (91.1%) patients with available clinical/molecular/laboratory data required for IPSS/DIPSS/MYSEC-PM assessment at diagnosis and at ruxolitinib start were included in the present analysis. Patients were enrolled into the JUMP trial (ClinicalTrials.gov Identifiers: NCT01493414) (n. 250) or within a compassionate use program (n.19). The remaining patients were treated off-study as per standard clinical practice.

Diagnosis of PMF and post-ET/ post-PV MF was made according to the WHO 2008²⁴ or the International Working Group on Myelofibrosis Research and Treatment (IWG-MRT) criteria,⁸ respectively. Bone marrow fibrosis was graded in accordance to the European Consensus Grading System.²⁵ Evolution into blast phase was diagnosed according WHO classification.¹ Molecular tests for detection of *JAK2*, *MPL* and *CALR* mutations and cytogenetic analysis were made as described elsewhere.²⁶ Ruxolitinib was administrated according to prescribing information. Spleen and symptoms' responses have been defined according to IWG-MRT/ELN criteria.²⁷ Symptoms' response was assessed by changes in Myeloproliferative Neoplasm Symptom Assessment Form total symptoms score (TSS).²⁸ The study was approved by the Institutional Review Board of each Institution and was conducted according to the Helsinki declaration. Clinical and laboratory parameters were evaluated both at diagnosis and at ruxolitinib start.

Statistical analysis

Categorical variables have been summarized by their median and range, and categorical variables by count and relative frequency (%) of each category. Comparisons of continuous variables between groups of patients were carried out by Wilcoxon-Mann-Whitney rank-sum test or Student's t-test, and association between categorical variables (2-way tables) was tested by the Fisher exact test or χ^2 , as appropriate. Also Logistic Regression was used (in univariate and multivariate analysis) in order to find the best fitting model to describe the relationship between the dichotomous dependent variable of interest and sets of independent (both binary and continuous) variables. Where Logistic Regression was used, Odds Ratios (OR) for the independent variables were specified. Multivariable logistic regression analysis was conducted on variables with $p < 0.05$ at univariate analysis. To avoid the issue of multicollinearity, which occurs when there are high correlations among predictor variables, leading to unreliable and unstable estimates of regression coefficients, a common solution is to remove highly correlated predictors from the model, because they supply redundant information. Therefore, before proceeding from univariate to multivariable analysis, collinearity amongst variables was detected by means of Pearson correlation test and variables that were found to be associated with the other factors were therefore excluded from the analysis. Overall survival (OS) was calculated by Kaplan-Meier function from the time of MF diagnosis or from ruxolitinib start to death or last contact, whichever came first, and compared by log-rank test. When calculating OS from the start of Ruxolitinib, the survival analysis was adjusted for left truncation ("delayed entry"). All reported p values are two-sided, and p values < 0.05 were considered statistically significant. All statistical analysis were performed with STATA15.

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RESULTS

Risk categories at MF diagnosis according to the International Prognostic Score System and to the MYSEC-Prognostic Model

A total of 421 MF patients were evaluable both for IPSS/MYSEC-PM at diagnosis and for DIPSS/MYSEC-PM at ruxolitinib start and were included in the analysis: 235 (55.8%) PMF patients and 186 secondary MF (post-PV MF: 117 patients, 62.9%).

At diagnosis, among the 235 patients with PMF, 44 (18.7%) were at low, 64 (27.2%) at intermediate-1 risk, 72 (30.6%) were at intermediate-2 risk and 55 (23.4%) were at high risk according to the IPSS. The IPSS could identify four categories of patients with significantly different survival, although the distinction between low and intermediate-1 categories was not fully evident. Specifically, OS at 4 years was: 97.4% (95% CI: 82.7-99.6), 96.5% (95% CI: 86.6-99.1), 83.2% (95% CI: 70.8-90.6) and 47.8% (95% CI: 28.9-64.4), respectively in low, intermediate-1, intermediate-2 and high-risk patients (*log-rank* $p < 0.001$).

Among the 186 post-PV/post-ET MF patients, the use of IPSS did not allow accurate risk stratification (**Figure 1A**). According to the MYSEC-PM, 20 (10.8%) patients were at low, 93 (50.0%) at intermediate-1 risk, 52 (27.9%) were at intermediate-2 risk and 21 (11.3%) were at high risk. The MYSEC-PM could effectively assess prognosis in post-PV/post-ET MF patients (*log-rank* $p < 0.001$). (**Figure 1B**).

Risk categories at ruxolitinib start according to the Dynamic International Prognostic Score System and to the MYSEC-Prognostic Model

At the start of ruxolitinib, patients' prognosis was re-evaluated according to the DIPSS (in PMF patients) and to the MYSEC-PM (in post-PV/post-ET MF patients). Since the time elapsed between diagnosis and RUX start might impact on OS post RUX, survivals from ruxolitinib start were adjusted for left truncation.

Considering the 235 PMF patients, 87 (37%) were at intermediate-1 risk, 136 (57.9%) were at intermediate-2 risk and 12 (5.1%) were at high risk according to the DIPSS. Overall survival at 2 years was: 93.7% (95% CI: 85.5-97.3), 70.3% (95% CI: 60.0-78.5) and 41.1% (95% CI: 6.8-74.6), respectively in intermediate-1, intermediate-2 and high-risk PMF patients (*log-rank* $p < 0.001$).

Importantly, the DIPSS could not correctly stratify patients with post-PV/post-ET MF in the 3 categories at different survival probability, since survival probability was comparable in intermediate-2 and high risk patients (Figure 1C). Among the 186 post-PV/post-ET MF patients, 12 (6.4%) were at low, 84 (45.2%) at intermediate-1 risk, 58 (31.2%) were at intermediate-2 risk and 32 (17.2%) were at high risk according to the MYSEC-PM. Conversely to the DIPSS score, the MYSEC-PM in post-PV/post-ET MF patients significantly stratified the survival of all 4 risk categories (*log-rank* $p < 0.001$) (Figure 1D).

Concordance rate between IPSS/DIPSS and MYSEC-PM at ruxolitinib start

When using the MYSEC-PM at diagnosis, 109 out of 186 patients (58.6%) with post-PV/post-ET MF shifted to a different risk category with respect to IPSS, with a concordance rate between the two scores ranging between 25% and 62% (**Figure 2A**). Specifically, 65.6% of patients included in the low-risk category according to IPSS moved to a higher risk category, while 75% of IPSS-defined high risk patients downgraded to a lower risk. Patients at intermediate-2 IPSS risk were very frequently reassessed as intermediate-1 risk (48.8%), with only 10.5% of the patients shifting to the high risk category. The concordance rate between DIPSS and the MYSEC-PM at ruxolitinib start was higher than that observed at MF diagnosis, ranging between 45.6% and 66.7%. Particularly, while 22.2% of patients at intermediate-1 DIPSS risk shifted to a higher risk category (high-risk, 4.5% of the cases), DIPSS-defined intermediate-2 risk patients were re-classified in a lower risk category in 32.9% of the cases (**Figure 2B**).

Comparison between primary and post-PV /post-ET myelofibrosis

According to the univariate logistic regression analysis, at diagnosis, patients with secondary MF were significantly older and carried less frequently anemia compared to PMF patients; also, the *JAK2*^{V617F} mutation was significantly more frequently detected. Additionally, leukocyte count was higher and palpable splenomegaly was more frequently detected by palpation in secondary MF. Notably, time from MF diagnosis to ruxolitinib start was longer in PMF compared to post-PV/post-ET MF patients. Multivariable logistic regression analysis was conducted on variables with $p < 0.05$ at univariate analysis: time from MF diagnosis to ruxolitinib start, spleen size, age, leukocyte count, and hemoglobin < 10 g/dl. Results show that, in multivariable analysis, Hemoglobin < 10 g/dl ($p = 0.006$) and time from MF diagnosis to Ruxolitinib start ($p = 0.002$) remain significant, while age ($p = 0.12$), spleen size ($p = 0.25$), and leukocyte count ($p = 0.28$) lose statistical significance (**Table 1**). Median follow-up from MF diagnosis was 50.6, 39.6 and 42.9 months in PMF, post-PV MF and post-ET MF, respectively ($p < 0.001$). At last contact, 88 patients (20.9%) had died, and 28 (6.6%) had developed a blast phase.

Compared to post-PV MF, patients with post-ET MF showed a higher platelet count, but lower hemoglobin level and leukocyte count at diagnosis. Also, post-ET MF patients presented less frequently a large (≥ 10 cm below left costal margin, LCM) splenomegaly and the *JAK2*^{V617F} positivity. The time from post-PV/post-ET MF diagnosis to ruxolitinib start was comparable in PPV and post-ET MF (**Table 2**). Notably, post-PV MF patients started ruxolitinib with larger splenomegaly and also with a slightly higher symptoms burden as assessed by the TSS. Median ruxolitinib exposure was 17.7, 15.2 and 21 months in PMF, post-PV MF and post-ET MF, respectively ($p = 0.65$).

Response to ruxolitinib according to type of MF diagnosis and to risk category

An IWG-MRT-defined spleen response was achieved by 100 (27.2%) and 116 (35.0%) out of 368 and 331 evaluable patients at 3 and 6 months, respectively. The rate of spleen responses were comparable in PMF and post-PV/post-ET MF patients at both time-points (28.3% vs 25.8% at 3 months, $p = 0.6$; 34.2% vs 36.0% at 6 months, $p = 0.7$). Overall, 299 (74.6%) and 281 (83.1%) out of 401 and 338 evaluable patients at 3 and 6 months, respectively, achieved a symptoms response. Analogously to spleen responses, also symptoms responses were comparable in PMF and post-PV/post-ET MF, specifically: 77.1% vs 71.3% at 3 months, $p = 0.2$; 84.7% vs 80.1% at 6 months, $p = 0.4$). Responses were also comparable between PPV and post-ET MF patients at each time-points. However, the 12-week titrated dose of ruxolitinib was lower in post-ET patients compared to post-PV MF patients (28.6 mg/day vs 38.1, $p = 0.006$).

Finally, we evaluated the correlation between DIPSS (in PMF) / MYSEC-PM (in post-PV/post-ET MF) and responses to ruxolitinib at 3 and 6 months (**Table 3**). PMF patients at DIPSS intermediate-1 risk showed a significantly higher rate of spleen responses at 3 months compared to intermediate-2/high risk patients. Analogously, post-PV/post-ET MF patients at MYSEC-PM low/intermediate-1 risk had a significantly higher rate of spleen responses compared to higher risk categories. Notably, by classifying post-PV/post-ET MF patients according to the DIPSS risk, the same association between responses and risk score was observed.

Toxicity to ruxolitinib according to type of MF diagnosis and to risk category

A total of 281 (67.5%) and 203 (56.4%) patients out of 416 and 360 evaluable patients developed grade ≥ 2 anemia at 3 months and at 6 months, respectively. Grade 3 anemia was observed in 43.7% and 36.9% at 3 and 6 months, respectively. No G4 anemia was registered. The incidence of grade 2-3 anemia was significantly higher in PMF compared to post-PV/post-ET MF patients at 3 and 6 months (72.2% vs 61.5% $p = 0.02$ and 61.8% vs 49.4%, $p = 0.02$, respectively).

A total of 179 (43.2%) and 158 (44.2%) patients out of 414 and 357 evaluable patients developed grade ≥ 2 thrombocytopenia at 3 months and 6 months, respectively. Two patients had a G4 thrombocytopenia. The incidence of grade ≥ 2 thrombocytopenia was comparable in PMF and post-PV/post-ET MF patients at 3 and 6 months ($p = 0.07$ and $p = 0.2$, respectively). Finally, patients at DIPSS/MYSEC-PM defined low/intermediate-1 risk showed a significantly

lower rate of drug-induced anemia and thrombocytopenia at 3 and 6 months compared to intermediate-2/high risk patients (**Table 3**).

DISCUSSION

MF post PV and ET has been generally considered superimposable to PMF in terms of prognosis and therapeutic approach.

However, over the last few years increasing evidence has shown that post-PV/post-ET MF should be considered as a distinct clinical entity⁹ and that the prognostic models used in PMF (IPSS, DIPSS, DIPSS-plus) may have a lower accuracy in secondary MF.²⁹ In order to evaluate clinical characteristics and performance of MYSEC-PM in post-PV/post-ET MF, we analyzed the outcome of a large cohort of MF patients (55.8% PMF, 27.8% post-PV MF and 16.4% post-ET MF) homogeneously treated with ruxolitinib in 23 European Hematology Centers.

The first result of the study is that the MYSEC-PM confirmed its predictive role in post-PV/post-ET MF patients while the IPSS failed to correctly stratify these patients at diagnosis. Also, the proportion of patients assigned to each risk category by the MYSEC-PM in the present series was similar to that reported by Passamonti et al. and also by the Spanish registry. However, in our experience PMF patients at intermediate-2 IPSS risk showed a survival probability superior to that generally reported in this specific risk category. Larger prospective cohorts are warranted to clarify whether the present observation is due to a selection of patients whose survival, regardless of risk category, was long enough to allow ruxolitinib therapy, to small sample size or to an improved therapeutic strategy in this setting. We indeed acknowledge that the retrospective nature of the study and inclusion criteria may have selected MF patients with specific disease characteristics not applying to the whole population. On the other hand, this cohort may represent the optimal setting to test prognostic models, since the homogeneity of treatment may have significantly reduced the differences in outcome related to different therapeutic approaches.

Additionally, the use of MYSEC-PM after post-PV/post-ET MF diagnosis, despite not recommended, will likely become shortly the standard routine practice, due to the lack of dynamic scores for secondary MF. Unlike the DIPSS that failed to confirm its predictive value in post-PV/post-ET MF patients, the MYSEC-PM, at ruxolitinib start, maintained a significant predictive value in secondary MF, proving to be a potential reliable prognostic tool also when used over time. These data, that require further validation, may suggest that the use of the MYSEC-PM could be extended also for follow-up evaluations.

The second result of this analysis is that both responses and toxicity rates were influenced by risk category. By applying the DIPSS and the MYSEC-PM at ruxolitinib start, patients at intermediate-1 risk achieved significantly higher spleen responses and lower hematological toxicities compared to higher risk patients. These findings are in line with our previous observations, pointing out that patients treated with a lower burden of the disease may achieve better therapeutic results.²³

Finally, the concordance rate of IPSS and MYSEC-PM at disease diagnosis was relatively low, with 41.8% (78 out of 186) post-PV/post-ET MF patients downgraded to a lower risk category and 13.6% (31 out of 186) upgraded to a higher risk category when using the MYSEC-PM. This finding may have significant practical implications, including both ruxolitinib and transplant indication. Indeed, allogeneic transplant is strongly recommended in patients with higher-risk categories, although it may be considered in intermediate-1 patients carrying additional high-risk features, like transfusion-dependent anemia, adverse cytogenetics and/or high molecular risk mutations.³⁰ Downgrading risk score in many post-PV/post-ET MF patients, the MYSEC-PM may potentially reduce the number of secondary MF patients candidate to transplant, the only eradicated treatment strategy in MF. Whether the MYSEC-PM can be used similarly to the IPSS/DIPSS to establish the indication to transplant should require further validation in larger studies. Additionally, the current experience demonstrates that a small but not insignificant fraction (6.4%) of patients at low risk according to the MYSEC-PM carried a substantial burden of symptoms and/or a large splenomegaly that required

ruxolitinib therapy. This observation reinforces the concept that in routine clinical practice the indication to ruxolitinib therapy is based primarily on clinical needs, regardless of risk category.

The third result of the study is that post-PV/post-ET MF presents a more hyper-proliferative disease compared to PMF, with significantly higher leukocyte count, hemoglobin levels, and spleen enlargement at diagnosis. Compared to post-ET MF, post-PV MF had the highest degree of myeloproliferation, showing increased hemoglobin/leukocyte levels and larger splenomegaly, but lower platelet count. These features are in line with previous observations, showing that the proportion of patients with transfusion dependency was higher in PMF, leukocytosis and systemic symptoms were higher in post-PV MF, while thrombocytopenia was more frequent in PMF and post-PV MF.¹² Post-PV MF patients maintained these features over time, starting ruxolitinib treatment with a higher burden of disease compared to post-ET MF. However, post-PV MF patients achieved rates of spleen and symptoms responses comparable to post-ET MF, probably thanks to the maintenance of a higher 12-weeks titrated dose. Despite comparable response rates, PMF patients experienced a significantly higher incidence of anemia compared to secondary MF. This finding may suggest a more cautious therapeutic approach in patients with PMF that may possibly benefit from the use of lower starting ruxolitinib doses in order to decrease the rate of early-onset drug-induced anemia.

Overall, the present study contributes to increase the knowledge on disease phenotype and outcome in secondary MF patients, and validates the use of the MYSEC-PM in patients with post-PV/post-ET MF.

Authors' Contributions

F.P., G.A.P. and N.P. designed the study and wrote the paper; A.P., N.P., G.B. M.Br., E.A. , M.T. M.Bo., A.T. A.I., B.M., N.S., M.D., M.D. M.Be., M.Cr., F.C., C.B., G.B., G.A., R.L., A.I., L.S., D.P, D.C. collected data and gave final approval; D.B. and M.L.B.R. performed statistical analysis; D.R., A.C., G.S., F.D.R, F.A., R.M.L., F.H., M.Ca, L.C. N.V. gave final approval.

Declaration of interests

F.P., G.A.P, M.Ti., and A.Iu. acted as consultant and received honoraria from Novartis; G.B. honoraria from Novartis, Janssen Amgen; M.Br., E.A. and M.Bo. honoraria by Novartis, BMS, Pfizer, Incyte; A.Is. honoraria from Novartis, Gilead, Janssen; M.Cr. honoraria form Janssen, Novartis, BMS, Celgene; F.C. honoraria from Novartis, Incyte, Pfizer; R.L. honoraria from Novartis, Celgene, BMS, Janssen; M.Tr. honoraria from Novartis, Celgene, BMS, Janssen; F.A. honoraria from Gilead, Sigma-Tau, Astellas, Roche; R.M.L. honoraria from Gilead, Novartis, Sanofi, Milteny.

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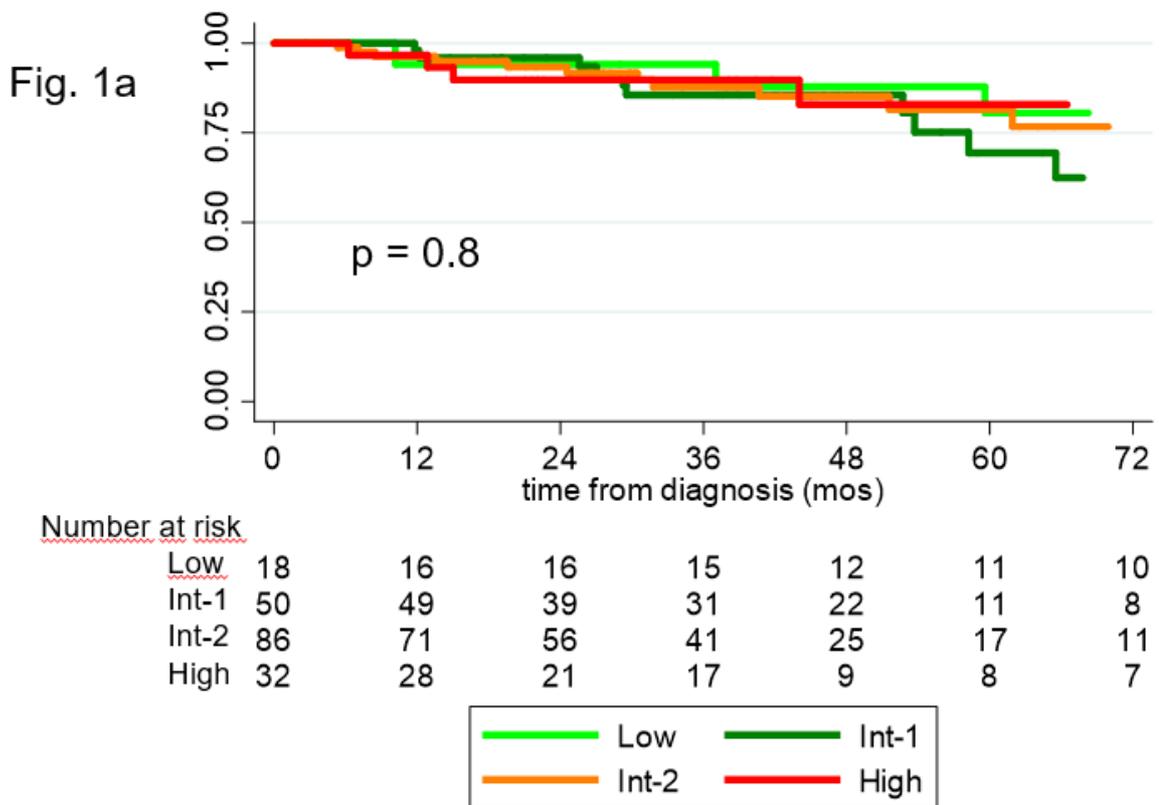
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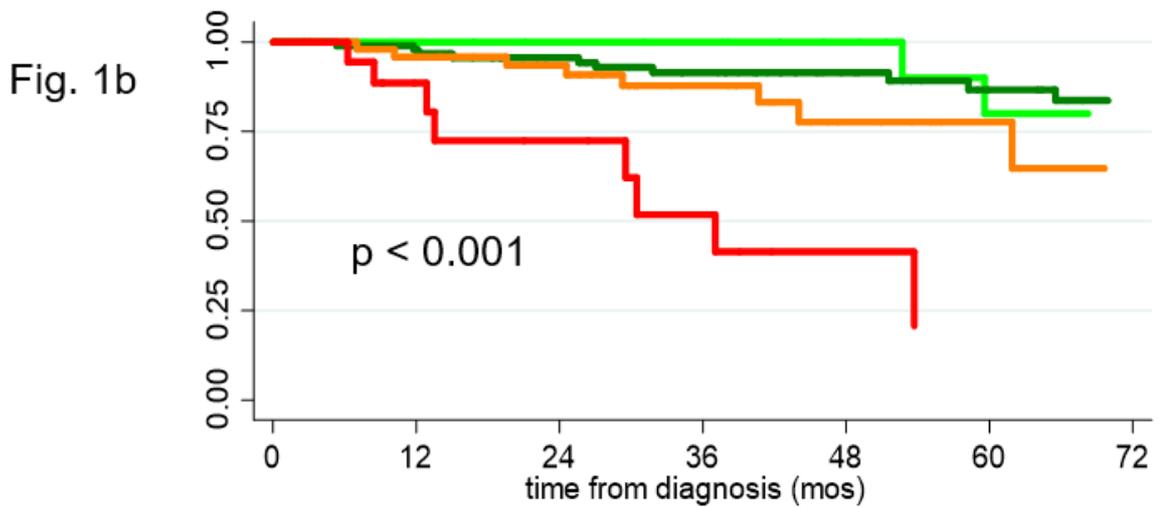
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Figure 1. Overall survival of patients with myelofibrosis post polycythemia vera and post essential thrombocythemia according to IPSS (fig.1a) and to MYSEC-PM (fig.1b) evaluated at diagnosis and according to DIPSS (fig.1c) and to MYSEC-PM (fig.1d) evaluated at ruxolitinib start and adjusted for left-truncation.

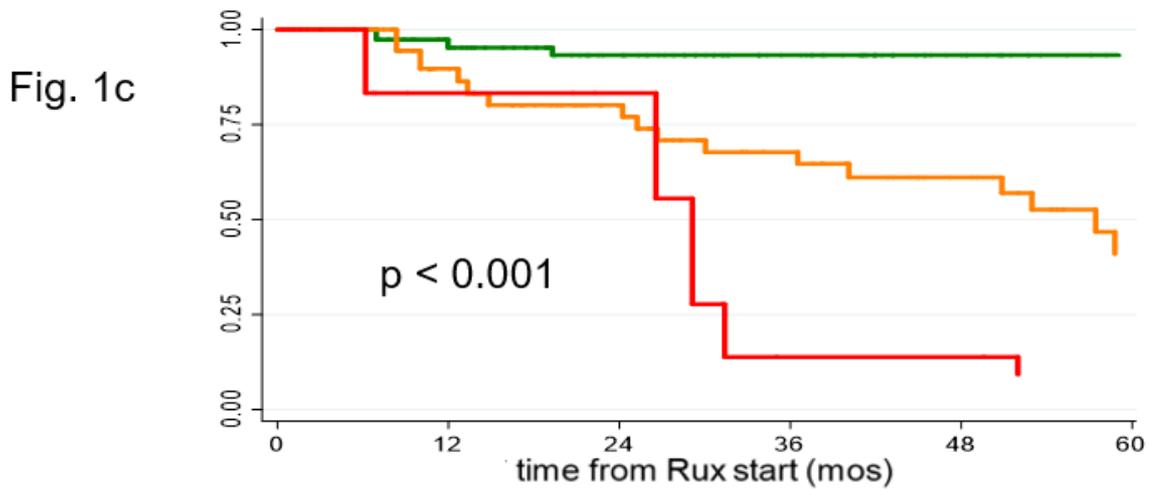
Figure 1A) OS at 4 years was: 87.8% (95% CI: 59.5-96.8), 85.5% (95% CI: 70.4-93.3), 85.2% (95% CI: 72.8-92.2) and 82.9% (95% CI: 58.2-93.7), respectively in low, intermediate-1, intermediate-2 and high-risk post-PV/post-ET MF patients (*log-rank p*=0.80). **Figure 1B)** OS at 4 year was 100%, 91.4% (95% CI: 82.7-95.8), 77.6% (95% CI: 56.3-89.4) and 41.4% (95% CI: 13.6-67.7), respectively in low (20 patients), intermediate-1 (93 patients), intermediate-2 (52 patients) and high-(21 patients) risk patients. **Figure 1C)** OS at 2 years was: 100%, 97,2% (95% CI: 89.2-99.3), 77.6% (95% CI: 60.7-87.9) and 47% (95% CI: 24.4-66.8), respectively in low, intermediate-1, intermediate-2 and high-risk patients (*log-rank p*<0.001). **Figure 1D)** OS at 2 years was: 100%, 97% (95% CI: 88.7-99.3), 72.6% (95% CI: 55.1-84.2) and 35.1% (95% CI: 13.6-57.7), respectively in low (12 patients), intermediate-1 (84 patients), intermediate-2 (58 patients) and high-(32 patients) risk patients, (*log-rank p*<0.001).





Number at risk

Low	20	19	17	16	12	8	6
Int-1	93	89	72	60	43	32	25
Int-2	52	45	35	23	11	6	4
High	21	11	8	5	2	1	1

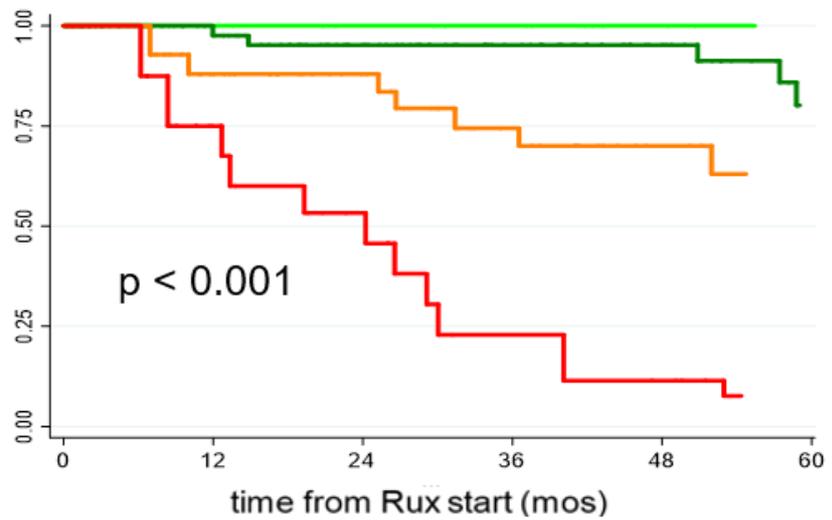


Number at risk

Int-1	90	65	51	28	6	0
Int-2	79	49	32	19	2	2
High	17	6	3	1	0	0



Fig. 1d

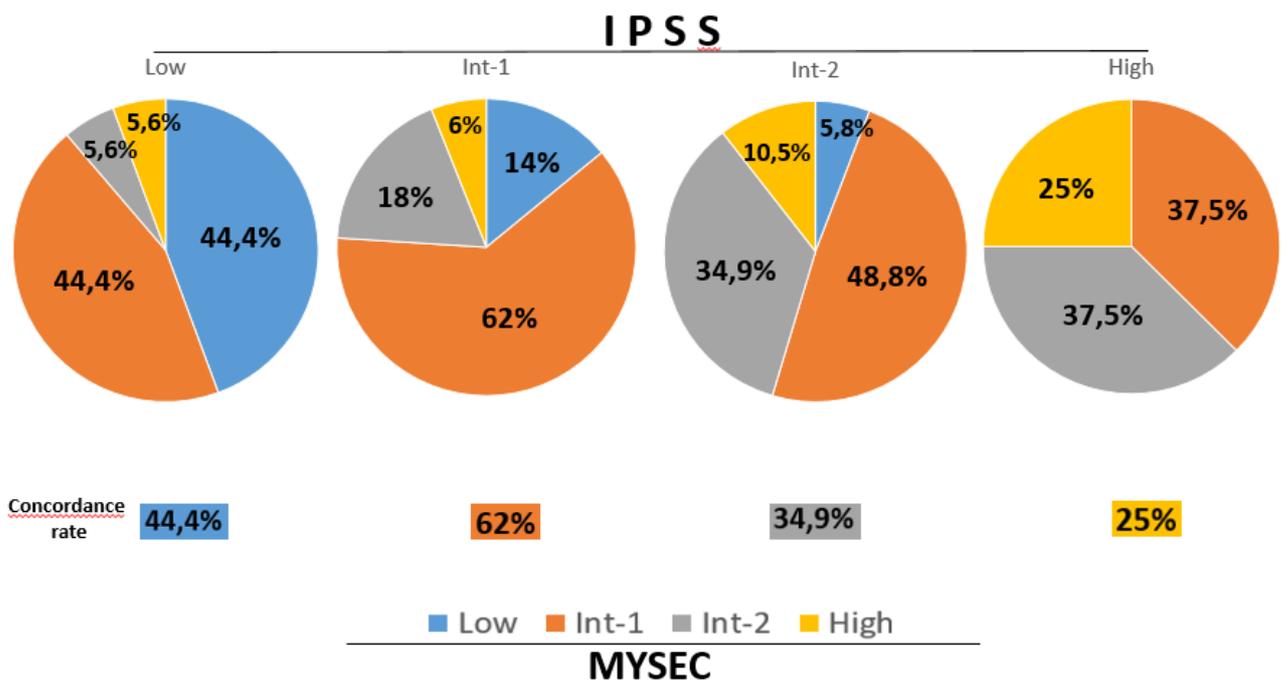


Number at risk

Low	12	9	9	7	1	0
Int-1	84	71	55	31	5	1
Int-2	58	39	24	15	3	1
High	32	15	7	2	1	0



Figure 2. Concordance between International Prognostic Score System (IPSS) and MYSEC-prognostic model at diagnosis (Figure 1a) and between the dynamic-IPSS and the MYSEC-PM at ruxolitinib start in patients with post-PV/post-ET MF



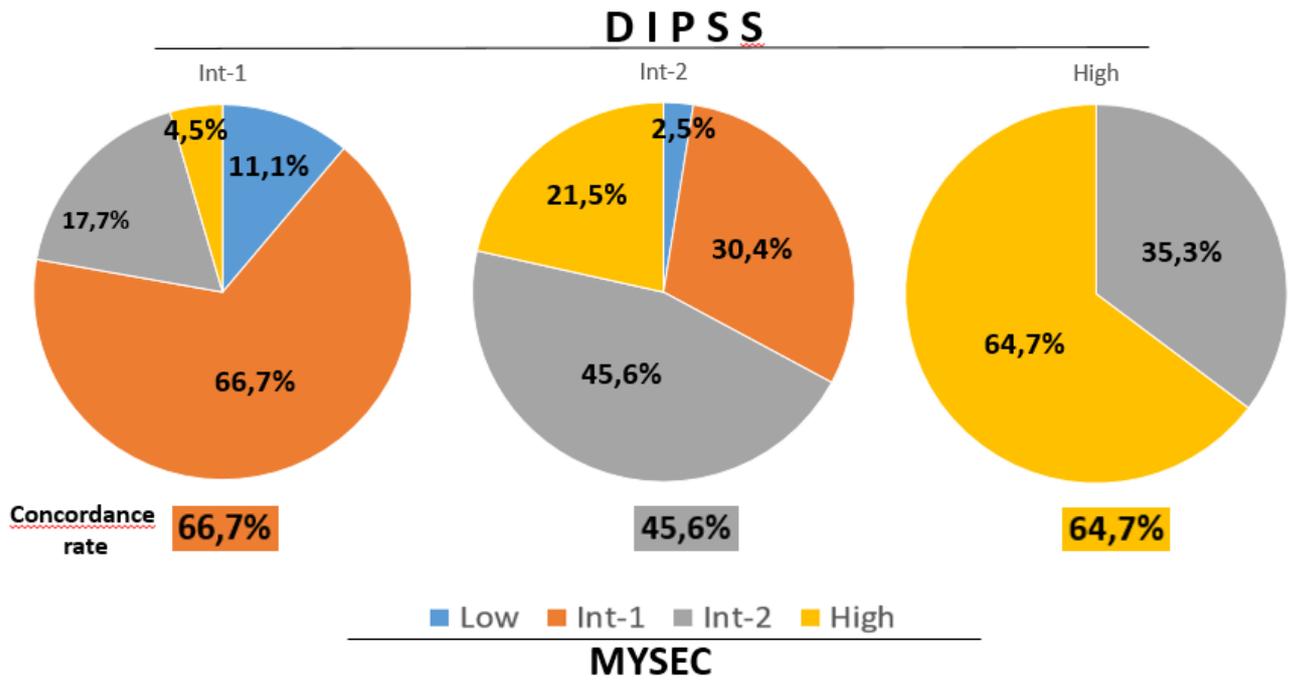


Table 1. Univariate and multivariate analysis of patients' characteristics at diagnosis according to the type of myelofibrosis (primary or post essential thrombocythemia/polycythemia vera myelofibrosis). After detecting collinearity amongst variables, multivariable logistic regression analysis was conducted on variables with $p < 0.05$ at univariate analysis.

Characteristics	n. (%) / Median (range)		UNIVARIATE		MULTIVARIATE	
			OR (95% CI)	<i>p</i> value	OR (95% CI)	<i>p</i> value
	PRIMARY MF (235)	Post-PV/post-ET MF (186)				
Male sex	146 (62.1%)	99 (53.2%)	1.44 (0.97-2.13)	0.07		
Median age years	63.4 (15.9-88.1)	65.8 (29.4-86)	0.97 (0.96-0.99)	0.004	1.01 (1.00-1.04)	0.12
Age > 65 years	101 (43%)	101 (54.3%)	0.63 (0.43-0.93)	0.02		
Median hemoglobin g/dl	11.5 (4.6-16.6)	11.4 (6.4-16.6)	0.96 (0.89-1.04)	0.4		
Hemoglobin <10 g/dl	83 (35.3%)	44 (23.7%)	1.76 (1.14-2.71)	0.01	0.52 (0.33-0.83)	0.006
Transfusion dependence	80 (34%)	39 (21%)	1.94 (1.24-3.03)	0.003		
Leukocytes count, $\times 10^9 / l$	9.3 (2.7-64)	11.8 (2.5-57)	0.97 (0.95-0.99)	0.01	1.01 (0.99-1.03)	0.28
Leukocyte $> 25 \times 10^9 / l$	18 (7.7%)	25 (13.4%)	0.53 (0.28-1.01)	0.05		
Platelet count, $\times 10^9 / l$	305 (35-1462)	343 (30-1521)	0.99 (0.99-1.001)	0.7		
Platelet $< 100 \times 10^9 / l$	19 (8.1%)	8 (4.3%)	1.96 (0.83-4.57)	0.1		
Platelet $> 200 \times 10^9 / l$	177 (75.3%)	148 (79.6%)	0.78 (0.49-1.24)	0.3		
Constitutional symptoms	118 (50.4%)	108 (58%)	0.73 (0.49-1.08)	0.1		
Palpable spleen	201 (86.3%)	173 (93%)	0.47 (0.24-0.93)	0.03	1.51 (0.74-3.08)	0.25
Spleen ≥ 10 cm	109 (46.8%)	89 (47.8%)	0.95 (0.65-1.41)	0.8		
Unfavorable karyotype	9 (7%)	9 (8.6%)	0.79 (0.30-2.07)	0.6		
$JAK2^{V617F}$ mutation	150 (75.4%)	155 (90.1%)	0.33 (0.18-0.61)	<0.001		
Median time from MF diagnosis to RUX, mos	25.8 (0.1-321)	11.1 (0-344.5)	1.01 (1.00-1.01)	<0.001	0.99 (0.98-1.00)	0.002
Time from MF diagnosis to RUX >2 yrs	121 (51.5%)	63 (33.9%)	2.07 (1.39-3.08)	<0.001		
Median follow-up from MF diagnosis, mos	50.6 (3.9-335)	41 (0.2-355.6)	1.01 (1.00-1.01)	<0.001		

Table 2. Clinical and laboratory parameters according to the type of secondary myelofibrosis (MF) at diagnosis and at ruxolitinib start.

Post-PV MF: Post Polycythemia Vera Myelofibrosis; Post-ET: Post Essential Thrombocythemia. OR: Odds ratio

Characteristics, n.(%) /median (range)	Post-PV MF (n.117)	Post-ET MF (n.69)	OR (95% CI)	<i>p value</i>
At diagnosis				
Male sex	68 (58.1%)	31 (44.9%)	0.58 (0.32-1.07)	0.08
Median age years	65.8 (29.4-86)	66.2 (38.9-82.5)	0.99 (0.96-1.03)	0.94
Age > 65 years	64 (54.7%)	37 (53.6%)	0.96 (0.52-1.74)	0.88
Median hemoglobin g/dl	12.2 (7-16.6)	10.3 (6.4-15.6)	0.67 (0.56-0.79)	<0.001
Hemoglobin <10 g/dl	14 (12%)	30 (43.5%)	5.66 (2.71-11.79)	<0.001
Median Leukocyte count, x10 ⁹ /l	12.5 (2.8-57)	9.5 (2.5-55)	0.97 (0.94-1.01)	0.11
Leukocyte>25x10 ⁹ /l	19 (16.2%)	6 (8.7%)	0.49 (0.19-1.30)	0.15
Median Platelet count, x10 ⁹ /l	328 (30-1521)	415 (100-1247)	1.002 (1.001-1.003)	0.006
Platelet >200 x10 ⁹ /l	87 (74.4%)	61 (88.4%)	2.63 (1.12-6.12)	0.02
Palpable spleen	112 (95.7%)	61 (88.4%)	0.34 (0.11-1.08)	0.06
Spleen ≥10 cm	67 (57.3%)	22 (31.9%)	0.35 (0.19-0.65)	0.001
Constitutional symptoms	73 (62.4%)	35 (50.7%)	0.62 (0.33-1.13)	0.12
Unfavorable karyotype	7 (10.9%)	2 (5%)	0.43 (0.08-2.17)	0.29
JAK2 ^{V617F} mutation	108 (98.2%)	47 (75.8%)	0.06 (0.01-0.26)	< 0.001
JAK2 ^{V617F} ≥50%	68 (73.9%)	15 (50%)	2.83 (1.21-6.65)	0.01
At RUX Start				
Median age, years	68.9 (44.7-88.2)	69.4 (44.5-83.4)	0.99 (0.96-1.03)	0.80
Age > 65 years	77 (65.8%)	44 (63.8%)	0.9 (0.49-1.70)	0.78
Median hemoglobin g/dl	12.1 (5.8-16.7)	9.7 (7.6-15.1)	0.72 (0.62-0.84)	<0.001
Hemoglobin <10 g/dl	29 (24.8%)	39 (56.5%)	3.94 (2.09-7.44)	<0.001
Transfusion dependence	22 (18.8%)	17 (24.6%)	1.41 (0.68-2.89)	0.35
Leukocyte count, x10 ⁹ /l	11.6 (1.3-155)	10 (2.5-85.2)	0.99 (0.98-1.01)	0.74
Leukocyte>25x10 ⁹ /l	18 (17.8%)	10 (15.1%)	0.82 (0.35-1.91)	0.65
Platelet count, x10 ⁹ /l	273 (52-1425)	320 (53-1887)	1.01 (1.0-1.01)	0.02
Platelet <100 x10 ⁹ /l	12 (10.3%)	2 (2.9%)	0.26 (0.06-1.2)	0.08
Platelet >200 x10 ⁹ /l	74 (63.2%)	58 (84.1%)	3.06 (1.45-6.46)	0.003
Palpable spleen	114 (97.4%)	66 (95.6%)	0.58 (0.11-2.95)	0.51
Spleen ≥10 cm	75 (64.1%)	33 (47.8%)	0.51 (0.28-0.93)	0.03
Median Total Symptoms Score (TSS)	20 (0-90)	20 (5-100)	0.99 (0.97-1.01)	0.52
TSS >20	47 (40.2%)	24 (34.8%)	0.79 (0.43-1.47)	0.46
Median Body Mass Index (BMI)	23.5 (15.6-31.6)	23.4 (18.4-30.9)	0.99 (0.9-1.09)	0.9
BMI<21	18 (18.6%)	15 (26.8%)	1.60 (0.73-3.51)	0.2
Grade 3 marrow fibrosis	30 (28.3%)	20 (30.3%)	1.01 (0.56-2.16)	0.78
Median Time from MF diagnosis to RUX start, mos	11.03 (0-344.5)	11.2 (0-213.6)	0.99 (0.99-1.01)	0.96
Time from MF diagnosis to RUX start >2 years	39 (33.3%)	24 (34.8%)	1.07 (0.56-1.99)	0.84

Table 3. Response and toxicity to ruxolitinib according to risk category at ruxolitinib start.

Responses and Toxicity, n. (%)	PRIMARY MYELOFIBROSIS			Post-PV/post-ET MYELOFIBROSIS		
	DIPSS category		<i>p value</i>	MYSEC-PM		<i>p value</i>
	Intermediate-1	Intermediate-2 & high		Low & intermediate-1	Intermediate-2 & high	
3 months						
Symptoms response	68 (82.9%)	104 (73.8%)	0.12	71 (74.7%)	56 (67.5%)	0.28
Spleen Response	29 (37.2%)	29 (22.8%)	0.03	29 (32.9%)	13 (17.3%)	0.02
Anemia grade ≥ 2	44 (51.2%)	125 (84.5%)	<0.001	44 (46.3%)	68 (78.2%)	<0.001
Thrombocytopenia grade 2/3	7 (8.1%)	29 (19.6%)	0.02	6 (6.4%)	11 (12.6%)	0.16
6 mos						
Symptoms response	64 (85.3%)	97 (84.3%)	0.85	75 (86.2%)	45 (73.8%)	0.06
Spleen Response	30 (41.1%)	33 (29.7%)	0.11	37 (44.1%)	16 (25.4%)	0.02
Anemia grade ≥ 2	31 (39.7%)	95 (75.4%)	<0.001	31 (35.8%)	46 (67.6%)	<0.001
Thrombocytopenia grade 2/3	5 (6.3%)	25 (20.2%)	0.01	10 (11.4%)	6 (8.9%)	0.62