Scientific section designations: Myeloid neoplasia

A clinical-molecular prognostic model to predict survival in patients with secondary myelofibrosis

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Key points

- *CALR* mutation is an independent predictor of favorable clinical outcome in patients with secondary myelofibrosis
- By integrating clinical and molecular data, MYSEC-PM improves the prognostication precision of secondary myelofibrosis

Abstract

Polycythemia vera (PV) and essential thrombocythemia (ET) are myeloproliferative neoplasms with variable risk of evolution into secondary myelofibrosis (SMF). While several prognostic models have been developed for primary myelofibrosis, no specific tools have been defined for risk stratification in SMF. To develop a prognostic model for predicting survival, we studied 685 *JAK2-, CALR-*, and *MPL*-annotated patients with SMF. Median survival of the whole cohort was 9.3 years (95% CI: 8-not reached). Through penalized Cox regressions, we identified the following negative predictors of survival to be included in the prognostic model: advanced age, hemoglobin level <11 g/dL, platelet count <150 x 10^9/L, circulating blasts ≥3%, *CALR*-unmutated genotype and presence of constitutional symptoms. Based on beta risk coefficients, we assigned 2 points to hemoglobin level, circulating blasts and *CALR*-unmutated genotype, 1 point to platelet count and constitutional symptoms, and 0.15 points to any year of age. We thereby constructed MYSEC-PM (Myelofibrosis Secondary to PV and ET-Prognostic Model): to assess risk category in the individual patient, we created an ad hoc nomogram. MYSEC-PM allocated SMF patients into four risk categories with different survival ($P < 0.0001$): low (median survival not reached; 133 patients), intermediate-1 (9.3 years, 95% CI: 8.1-not reached; 245 patients), intermediate-2 (4.4 years, 95% CI: 3.2-7.9; 126 patients), and high risk (2 years, 95% CI: 1.7-3.9; 75 patients). MYSEC-PM is an integrated clinical-molecular prognostic model able to identify different patterns of survival in SMF patients, and represents a useful tool for decision-making in both clinical and trial settings.

INTRODUCTION

Polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF) are the classical BCR/ABL1-negative myeloproliferative neoplasms (MPN).[1,2] The two more indolent diseases, PV and ET, nevertheless, can progress to secondary myelofibrosis (SMF), named post-PV (PPV) MF and post-ET (PET) MF,[3] and to blast phase (BP),[4] that result in worsening survival.[5]

In clinical practice and in clinical trials, primary myelofibrosis (PMF) and SMF are considered similar. The IPSS (International Prognostic Scoring System)[6] and its time-dependent variants (Dynamic IPSS –DIPSS and DIPSS-plus)[7,8] are often used to predict survival and to plan therapy for SMF patients. However, these models have been developed in patients with PMF and are suboptimal to predict survival in SMF.[9-11] Recently, having acquired the prognostic implication of phenotype driver mutations and of additional mutations, the prognostication in MPN is moving towards integrated clinical-molecular models. [12-16] Treatment strategy of SMF is similar to that of PMF.[17] JAK inhibitors[18] are equally effective in PMF and in SMF, however, an analysis of the COMFORT-2 study showed that a higher spleen response was obtained in PET-MF compared with PMF.[19]

The MYSEC (MYelofibrosis SECondary to PV and ET) project recently disclosed genotype-phenotype associations in the largest cohort of SMF patients published to date, including 685 patients.[20] We found that at presentation *JAK2*-mutated patients had higher white blood cell count and greater splenomegaly than *CALR*-mutated patients and that *CALR* type 1/type 1-like and *CALR* type 2/type 2-like were similar in terms of clinical presentation and outcome. Blast phase incidence was higher in *JAK2*-mutated PET MF and TN patients (triple negative, i.e. without *JAK2, MPL, CALR* mutations) when compared with *CALR*-mutated patients.

In this study based on the MYSEC database of 781 SMF patients, which has 685 patients molecularly annotated for phenotype driver mutations, we developed an integrated clinical-molecular model to predict survival of SMF. We call this the MYSEC-PM (Myelofibrosis Secondary to PV and ET-Prognostic Model).

PATIENTS AND METHODS

Study population

This study includes 781 patients collected from 16 international centers (Table 1S). All patients have demographic, clinical, and hematologic data at diagnosis and an adequate follow-up. No differences in disease presentation (white blood cell count, hemoglobin level, platelet count) were observed among centers applying the Kruskal Wallis and pairwise Wilcoxon rank-sum tests. Driver mutation status was requested as secondary objective. Diagnoses of PPV MF and PET MF were performed between 1981 to 2015 and were locally reviewed according to the International Working Group on Myeloproliferative Neoplasm Research and Treatment (IWG-MRT) criteria.[3] Evolution to BP was defined when leukemic blast cells were more than 20%, according to the World Health Organization (WHO) criteria.[21] The study was approved by the Institutional Review Board of each Institution and conducted in accordance with the principles of the Declaration of Helsinki.

Statistical analysis

Descriptive summaries are reported as median and range for continuous covariates, and count and relative frequency for categorical ones. Continuous baseline values were compared via non-parametric Mann-Whitney U tests; categorical feature counts were compared with Fisher's exact tests. Time-to-event analyses were performed via Kaplan-Meier curves, using log-rank tests for comparisons and semi-parametric Cox models for regression. Events were assumed to be death for any cause, censored at last follow-up or at the time of transplant, thrombosis and leukemia. P values <.05 (2-tailed) were considered significant. To select a parsimonious set of covariates on which to base the prediction algorithm, we fitted regularized regression models according to the least absolute shrinkage selection operator (LASSO) method, entering all the available discretized covariates. LASSO fits a sequence of models with varying degrees of penalization in order to shrink less-relevant coefficients to zero, thus effectively performing a variable selection.[22] The performance of the models was evaluated with 10-fold cross-validation; the highest shrinkage factor providing performance within one standard deviation of the optimal cross-validated one was selected.[23] Statistical analyses were performed using R version 3.3.2 and packages rms 4.5, survival 2.39, and glmnet 2.0.

RESULTS

Presenting features of SMF patients, comparison of PET MF and PPV MF

Overall, 781 SMF (397 PET MF, 384 PPV MF) were included in the study and followed for a median time of 3 years (range, 0.6-27.3). Demographics and clinical features of patients at onset of SMF are shown in Table 1. Patients with PPV MF were older, had higher values of white blood cells and hemoglobin, larger spleen size and lower platelet count than those with PET MF. Pearson pairwise test demonstrated that at diagnosis patients with PPV MF had significantly higher frequency of constitutional symptoms, abnormal karyotype and prior thrombosis than those with PET MF. A significantly higher number of PPV MF patients had received cytoreductive treatments (281 with PET MF, 313 PPV MF, $P < .001$).

Events during the course of SMF

Incidence rates of events are reported in Table 2. For their calculation we took into account death and stem cell transplant as competing risks with thrombosis and leukemia. In detail, thrombotic events occurred in 98 SMF (12.5%; 52 PET MF and 46 PPV MF), blast phase in 66 SMF (8.4%, 39 PET MF and 27 PPV MF) and death in 220 SMF (28.1%, 99 PET MF and 121 PPV MF). Cause of death was known in 171 of the 220 patients who died: non-clonal disease progression in 65 (38%), blast phase in 57 (33%), second malignancy in 13 (8%), infection in 14 (8%), heart failure in 12 (7%), vascular complications in nine (5%), and other in one (1%). Median survival was 11.6 years (95% CI: 8-NR) in PET MF and 7.4 years (95% CI: 6.7-9.3) in PPV MF, without a resulting significant difference (Supplemental Figure 1, log-rank test, $P = .055$). We therefore analyzed the two SMF cohorts as one group.

Survival and identification of risk factors for survival

The prognostic model development was based on 685 molecularly annotated patients, whose median survival was 9.3 years (95% CI: 8-NR), as illustrated in Figure 1. To ascertain whether SMF survival has increased over calendar years, we performed a Cox regression including calendar year of diagnosis (as a linear covariate) correcting for IPSS risk category. We found that the trend of survival was not significantly changed ($P = .064$).

Thus, we first performed an exploratory univariate analysis developing Cox regression models considering each covariate separately. To account for possible nonlinear effects, restricted cubic

spline with 3 nodes were considered for continuous predictors. Single-variate Cox Proportional Hazards regression showed that advanced age, male gender, lower hemoglobin level, greater white blood cell count, lower platelet count, higher circulating blast count, bone marrow fibrosis grade 3 vs. grade 2, presence of constitutional symptoms (fever, weight loss, night sweats), history of thrombosis before SMF, longer time from ET/PV to SMF negatively affected survival (maximum *P* values = .004). Interestingly, a normal karyotype was associated with longer survival (*P* = .001), but, as cytogenetic data were available in only 340 patients (49%), we excluded this variable from the statistical analysis. Conversely, type of diagnosis (PET MF, PPV MF), centers, spleen and liver size were neutral for survival.

Analysis of cutpoints of continuous variables indicated marked differences for patients with white blood cell count higher than 25 $x10^9$/L, hemoglobin value lower than 11 g/dL, platelet count lower than 150 $x10^9$/L, circulating blast equal to or higher than 3% and time to SMF greater than 10 years (*P* < .0001 each). An exploratory multi-class regression showed that HRs (hazard ratios) for *CALR*-unmutated genotypes (i.e, *JAK2*-mutated, *MPL*-mutated and triple negative) had overlapping confidence intervals, and significantly different from *CALR*-mutated genotype (*P* = .003), thus determining a binary category (*CALR*-mutated vs. *CALR*-unmutated) for genotype. Multivariate models consistently showed age at diagnosis to be an important predictor for survival (P < 0.0001). In order to minimize information loss on this covariate, we retained age at diagnosis as a continuous covariate.

We then selected the significant covariates employing a least absolute shrinkage and selection operator (LASSO) Cox regression. At the selected value of the regularization parameter, λ = 0.053, six covariates remained with non-null coefficients: advanced age, hemoglobin level below 11 g/dL, platelet count below 150 $x10^9$/L, circulating blasts equal to or higher than 3%, *CALR*-unmutated genotype, presence of constitutional symptoms. We generated a final Cox regression model incorporating the identified covariates; the final model provided β coefficients, the corresponding hazard ratios and their 95% confidence intervals, which are reported in Table 3. All of the coefficients remained highly significant (*P* < .003); a test for Schönfeld residuals revealed no deviations from the proportional hazards assumption, except for a minor departure for constitutional symptoms.[24]

Development of the prognostic model

All factors shown in Table 3 were therefore included in the new prognostic model for SMF, named MYSEC-PM. To simplify the application of the risk score, we quantified the risk coefficients as integer risk points (Table 3). Namely, we allocated two points to hemoglobin level below 11 g/dL, circulating blasts equal to or higher than 3% and to *CALR*-unmutated genotype, one point to platelet count lower than 150 $x10^9$/L and to the presence of constitutional symptoms. Age-related risk was kept continuous and rescaled, yielding approximately 0.15 points per year.

We thus recoded the MYSEC-PM into four categories of adequate size by pooling consecutive score values. The resulting risk categories were: low-risk (score less than 11, 133 patients), intermediate-1 risk (score equal to or higher than 11 and lower than 14, 245 patients), intermediate-2 risk (score equal to or higher than 14 and less than 16, 126 patients) and high risk (score equal to or higher than 16, 75 patients). Survival was significantly different among the risk groups (Figure 2A, log-rank test $P < 10^{-6}$). Median survival was not reached in the low risk, 9.3 years (95% CI: 8.1-NR) in the intermediate-1 risk, 4.4 (95% CI: 3.2-7.9) in the intermediate-2 risk and 2 years (95% CI: 1.7-3.9) in the high risk category. Supplemental Figure 2 shows survival compared to age- and sex-matched U.S. population.

Taking low risk as reference, the estimated average HR for intermediate-1 risk was 3.6 (95% CI: 1.8-7.2), for intermediate-2 risk was 10.6 (95% CI: 5.3-21.1) and that for high risk was 29.1 (95% CI: 14.1-59.8). When used to assign patients to the four discrete risk categories, the test retained very good predictivity (cross-validated C statistics 0.78) and calibration.

How to use the prognostic model in clinical practice: the MYSEC PM nomogram

Given the hybrid nature (continuous age, discrete points) of the risk prediction model, we provide a discrete/continuous nomogram (Figure 2B) to interpolate the final score and assess the individual patient's risk in an easy manner. The MYSEC PM nomogram provides an at-a-glance diagram to combine the effect of age (continuous) and other covariates, at the same time providing color-coded read-outs on the resulting risk category. To calculate the MYSEC-PM doctors have to: 1) collect information on non-age prognostic variables (hemoglobin value, platelet count, circulating blast counts, constitutional symptoms, genotype), thus refer to Table 3 to assign the points and calculate their sum (score); 2) collect patient's age; 3) use the nomogram (Figure 2B) to locate the combination of score (read on the vertical axis) and age (on the horizontal axis) – the

color at the location indicates the final risk category, 3) estimate the individual survival on the Kaplan Mayer curve (Figure 2A). To further illustrate and expedite the use of the score, the nomogram is also made available as an interactive web application for desktop and mobile use (available online at https://tonigi.shinyapps.io/WebCalculator/).

Validation process of the MYSEC-PM

The predictive value on survival of the MYSEC-PM was verified computing Harrell's concordance index C, which yielded C = 0.79 on the input data set and C = 0.78 in 40-fold cross-validation (the C statistics indicates the fraction of subject pairs for which survival is predicted correctly relative to each other. The risk of overfitting is reduced by the LASSO covariate selection procedure outlined earlier, which employs a 10-fold cross-validation in the selection phase. We also measured the relative quality of survival stratification of the new MYSEC-PM by direct comparison with another prognostic models used in SMF, the IPSS.[6] The IPSS risk categories assigned at diagnosis did have good predictive value (C = 0.70), albeit lower than that of the SMF-specific model proposed here. Correspondingly, Akaike information criterion values amount respectively to 1416 and 1485 for the two scores. In summary, these results confirm that the discriminant power of the MYSEC-PM is very high, and provides better predictions than the IPSS model.

Mutation status distribution in the MYSEC-PM risk categories

Supplemental Figure 3 describes phenotype driver mutations in the four risk groups. Of interest, *CALR* mutations were absent in high risk patients.

DISCUSSION

Diagnosis of SMF is based on the IWG-MRT criteria, established in 2008: an antecedent WHO-based diagnosis of PV or ET including appropriate mutations and a bone marrow fibrosis above grade 1 are the two main criteria.[3] The molecular anatomy of PV and ET has changed from 2008, leading to the new WHO classification in 2016.[1] By enriching the MYSEC database with the phenotype driver mutations of the *JAK2, CALR* and *MPL* genes,[1,2] we provide a molecularly updated diagnosis of PV and ET and consequently of SMF. Concerning the accompanying mutations of MF,[1] no impact on SMF survival has been demonstrated,[25] differently from their effect in PMF.[12] The assessment of bone marrow myelofibrosis requires bone marrow biopsy. Our

study is representative of real-life in Europe and the United States: doctors perform bone marrow biopsy when they suspect disease evolution, an approach that remains a mainstay in recent recommendations.[26] Of note, the MYSEC database showed that the longer the span between PV/ET diagnosis and SMF, the worse the survival. This suggests to carefully monitor PV/ET patients in order to identify SMF evolution earlier, especially if disease-modifying treatments may be envisaged.

The MYSEC study also characterizes clinical phenotype and events of SMF. PPV MF and PET MF have substantial differences in clinical presentation, with a more "proliferative" phenotype in PPV MF, a pattern that is confirmed by the higher rate of PPV MF patients receiving cytoreductive agents. Of interest, the incidence of thrombosis ranged from 2.2 to 3.2 / 100 patients-year in PET MF and PPV MF, respectively, and accounted for 5% of deaths. These data clearly indicate that the risk of vascular complications is still significant in SMF. Perhaps, thromboprophylaxis should be considered in SMF, if not contraindicated because of a bleeding history or a low platelet count.

The median survival in SMF was 9.3 years without significant differences between PPV MF and PET MF. In the IPSS study of PMF patients the median survival was 6.5 years.[27] It is likely that there are some differences in terms of survival between SMF and PMF, but only an appropriate study can assess this point. The MYSEC dataset did not disclose any change of SMF survival over calendar years of diagnosis. This seems to suggest that treatment strategies have not changed the disease history yet. Modern approach to myelofibrosis treatment includes the use of JAK inhibition and allogenic stem cell transplantation (ASCT).[17] In PMF, we demonstrated that ruxolitinib might modify life expectancy in higher risk categories[18] and that ASCT improves survival in higher risk categories, with the opposite effect in low risk patients, when matched with a cohort of conventionally treated individuals.[28] As we excluded patients with SMF from these analyses, the effect of these strategies on survival is not known in SMF.

Concerning current risk stratification of patients with SMF, the IPSS[6] and DIPSS[8] prognostic models are used in clinical practice[26] as well as in clinical trials.[29-33] However, these models have been developed in patients with PMF and, as a consequence, their application outside that setting is arbitrary and not data-supported. Advanced age, hemoglobin level below 11 g/dL, platelet count below 150 x10^9/L, circulating blast cells equal to or greater than 3%, *CALR*-unmutated genotype

and the presence of constitutional symptoms are the risk factors composing the MYSEC-PM. Advanced age, anemia, circulating blast cells and the presence of constitutional symptoms are both components of the MYSEC-PM and the IPSS model,[6] and advanced age and constitutional symptoms also stratify patients at the time of ASCT for survival.[34] This indicates a role of these factors in myelofibrosis survival prediction in general.

Myelofibrosis is an age-related disease and advanced age is the most powerful prognostic factor for survival prediction. This is not surprising from a biological standpoint as hematopoietic stem cells are modified during aging influencing disease development and eventually favoring clonal hematopoiesis with selection of mutated cells.[35] It is noteworthy that the most frequently involved age-related somatic mutations (*DNMT3A, TET2, ASXL1,* and *JAK2*)[36] are also implicated in myelofibrosis development.[12]

The extended study of the three phenotype driver mutations helped to recognize the favorable impact on survival of *CALR* mutations,[20] and in this latter analysis *CALR*-unmutated genotypes (*JAK2*, *MPL*, triple negativity) are associated with a worse survival in multivariable analysis. The association of *CALR* mutations with a benign outcome in SMF, also highlighted by the absence of *CALR*-mutated patients within the MYSEC-PM high-risk group, remains to be determined. Although all phenotype driver mutations activate the JAK/STAT pathway, subtle changes in the activation mechanism have been described among mutants.[37] The molecular profiling of SMF patients allows the MYSEC-PM to improve risk stratification in SMF, as demonstrated by the superior accuracy in survival prediction of MYSEC-PM over IPSS.

The MYSEC-PM identifies four risk categories with different survival: median survival was not reached in the low risk, 9.3 years in the intermediate-1 risk, 4.5 years in the intermediate-2 risk and 2 years in the high risk category. This information may be directly translated into clinical practice to personalize treatment options. Young and fit patients with intermediate-2 and high risk disease can be considered candidates for ASCT on the basis of the European LeukemiaNet recommendations,[26] which give an indication for ASCT in MF patients with a life expectancy below five years. On the opposite, patients at low risk have an indolent disease and a more conservative approach seems reasonable. Patients at intermediate-1 risk should be discussed on an individual basis in SMF. Ruxolitinib can be offered on the basis of the national indication/reimbursement

rules since it has been intensively studied in SMF patients with intermediate and high risk disease according to clinical-based prognostic models.[30,31] Concerning investigative clinical trials, the use of MYSEC-PM in the selection of SMF patients may help in the identification of patients at higher risk who may be candidates for new treatment strategies or at lower risk who may be candidates for preventive approaches targeting disease progression/survival.

In conclusion, the MYSEC-PM is an integrated clinical-molecular prognostic model developed in 685 molecularly annotated SMF patients within a multi-institutional international collaboration in Europe and the United States. MYSEC-PM identifies different patterns of survival in patients with SMF and its use is facilitated by the specific nomogram. These observations are useful for clinical decision-making and for designing clinical trials.

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Authorship

Contribution: F.P., A.M.V, M.C., B.M., M.M. contributed to the conception of the work; F.P., T.G., B.M. contributed to the design of the work; T.G. performed the statistical analysis; F.P., B.M., P.G., M.C., M.M., A.R., M.C., R.K., J.G., J.J.K., F.C., T.D., N.V., V.DS., M.R., R.T.S., G.B., F.A., D.C., E.R., M.Me., D.P., T.B., L.P., A.M.V. contributed to the acquisition, analysis, or interpretation of data for the work; F.P., B.M., T.G., P.G., M.C., M.M., A.R., M.C., R.K., J.G., J.J.K., F.C., T.D., N.V., V.DS., M.R., R.T.S., G.B., F.A., D.C., E.R., M.Me., D.P., T.B., L.P., A.M.V. contributed to revising the manuscript critically for important intellectual content and approved the final version of the manuscript.

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Legend to Figures

Figure 1. Estimate of survival in 718 patients with secondary myelofibrosis. This represents the estimate of survival of the entire cohort of patients with secondary myelofibrosis.

Figure 2. The MYSEC-PM. (A) The MYSEC-PM estimate of survival in patients with secondary myelofibrosis molecularly annotated for *JAK2*, *CALR*, *MPL* mutations. Risk factors and relative points composing the MYSEC-PM are patient's age (0.15 per patient's year of age), hemoglobin level below 11 g/dL (2 points), platelet count lower than 150 $x10^9$/L (1 point), circulating blasts equal to or higher than 3% (2 points), presence of constitutional symptoms (1 point) and *CALR*-unmutated genotype (2 points). The final risk category is to be calculated with the MYSEC-PM nomogram (Figure 2B). The four risk categories are: low-risk (median survival not reached; 133 patients), intermediate-1 risk (median survival 9.3 years, 95% CI: 8.0-NR; 245 patients), intermediate-2 risk (median survival 4.5, 95% CI: 3.2-7.9; 126 patients) and high risk (median survival 2.0 years, 95% CI: 1.7-3.9; 75 patients) (2). (B) The MYSEC-PM nomogram. The MYSEC PM nomogram visually assigns the MYSEC-PM risk category starting from the non-age prognostic variables (vertical axis) and the patient's age (horizontal axis) illustrated in Table 3. To determine the risk category of an individual patient with hemoglobin value of 10 g/dL and circulating blast of 6%, for example, follow the horizontal line, starting from the non-age-parameter-sum of 4 on the vertical axis (see Table 3 for points) to the age of the patient and record the color at that point. If the patient is 40 years old, the 4-line and the vertical 40-year line cross in the green field, corresponding to the low risk category, while if the patient is 70 years old, the 4-line and the vertical 70-year line cross in the violet field, corresponding to the intermediate-2 risk category.

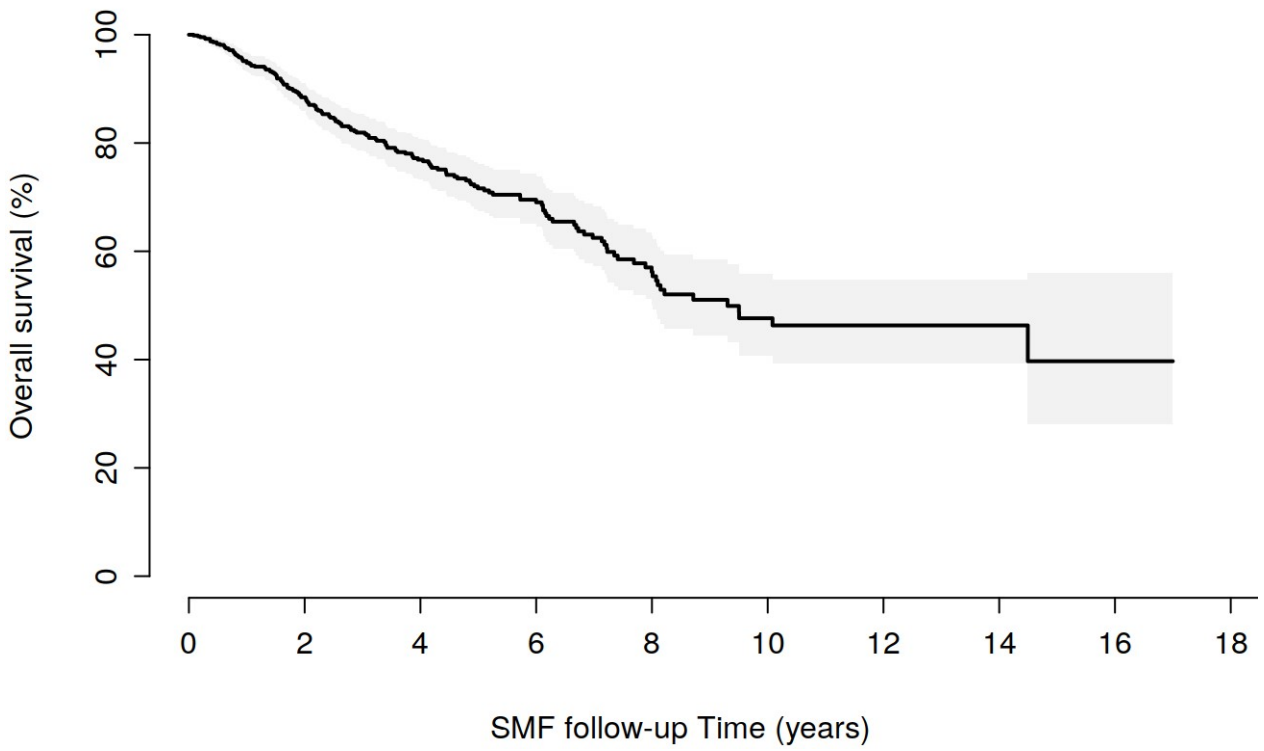

Figure 1

Figure 1: Estimate of survival in patients with secondary myelofibrosis.

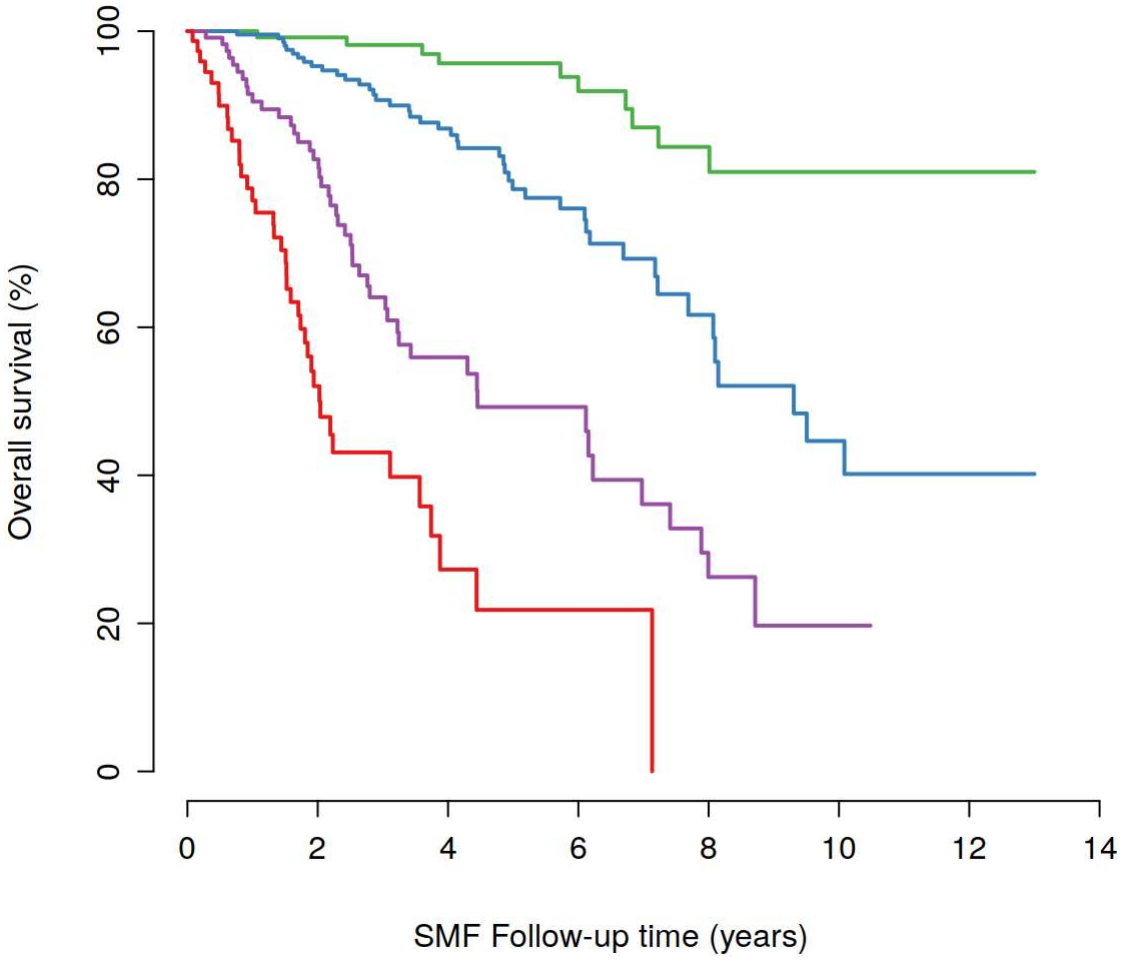

Figure 2A

Figure 2A: The MYSEC-PM estimate of survival in patients with secondary myelofibrosis molecularly annotated for *JAK2*, *CALR*, *MPL* mutations.

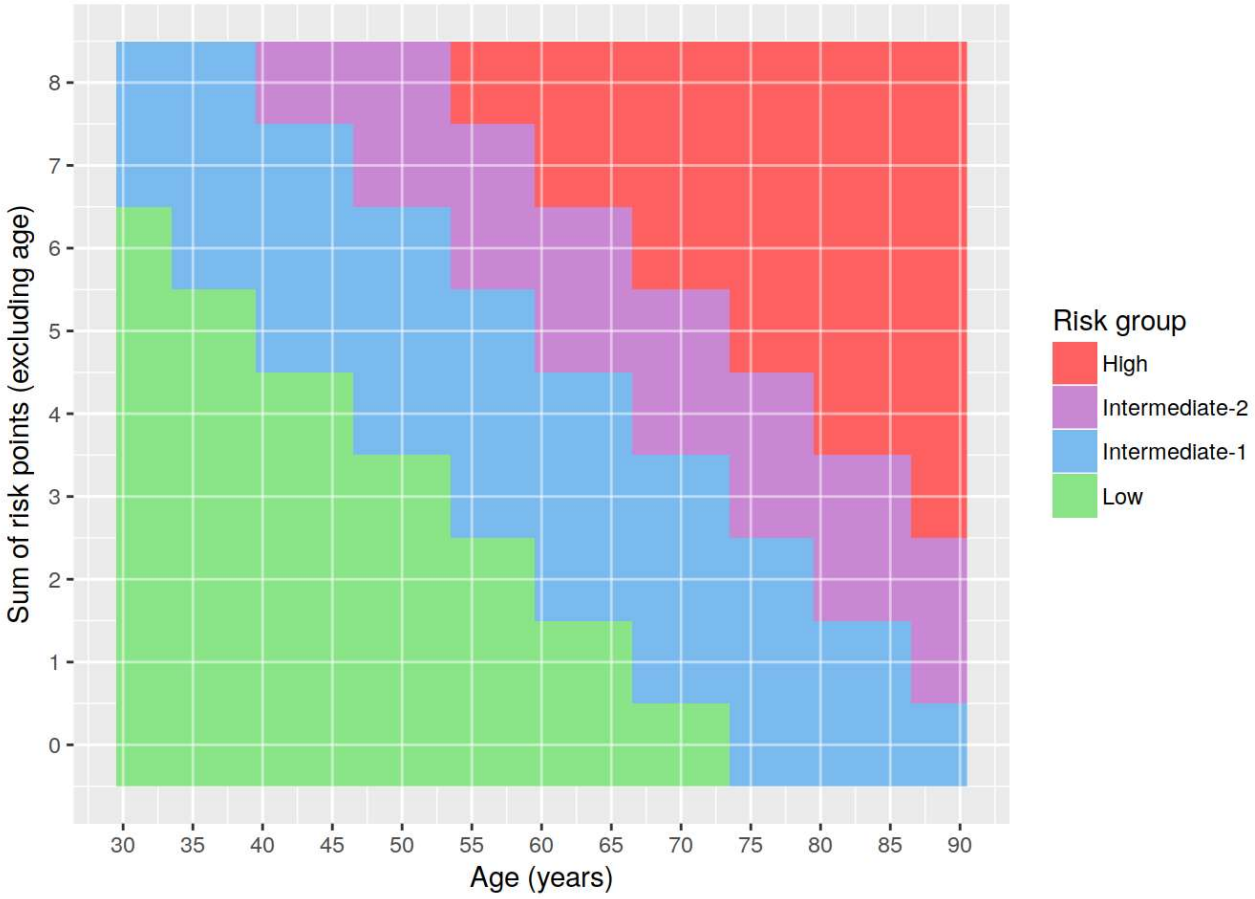

Figure 2B

Figure 2B: The MYSEC-PM nomogram.

Table 1. Hematological and clinical features of 781 patients with post essential thrombocythemia and post polycythemia vera myelofibrosis.

	SMF (n = 781)	PET MF (n = 397)	PPV MF (n = 384)	*P* value
Age, median (range), years	64 (25-96)	64 (25-93)	65 (34-96)	.01
Age older than 65 years, n. (%)	374 (48)	180 (45)	194 (51)	.15
Follow-up, median (range), years	3.0 (0.6-27.3)	3.1 (0.6-17.4),	2.8 (0.6-27.3)	.85
Time to SMF, years (range)	10.7 (0.1-41.4)	10.3 (0.3-39.3)	11.2 (0.1-41.4)	.17
History of cancer, n. (%)	94 (13)	43 (11)	51 (14)	.28
History of thrombosis, n. (%)	187 (25)	79 (21)	108 (29)	.01
Male gender, n. (%)	409 (52)	197 (50)	212 (55)	.13
WBC, median (range), $x10^9$/L	10.2 (1.1-98.4)	7.0 (1.1-97.3)	13 (1.7-98.4)	< .001
Hb, median (range), g/dL	11 (5-16)	10.6 (5-15.7)	11.9 (6.8-15.7)	< .001
PLT, median (range), x 10^9/L	336 (15-1908)	382 (25-1908)	290 (15-1689)	< .001
Circulating blast 3% or more (%)	72 (10)	36 (10)	36 (10)	.92
Spleen size,* median (range)	7 (0-34)	4 (0-27)	9 (0-34)	< .001
Constitutional symptoms, n. (%)	319 (44)	134 (37)	185 (51)	< .001
Normal karyotype,** n. (%)	248 (66)	136 (72)	112 (59)	.007
Favorable karyotype,** n. (%)	313 (87)	160 (88)	153 (86)	.58
JAK2 (V617F)	533 (78)	181 (54)	352 (100)	< .001
CALR	102 (15)	102 (31)	-	
MPL	30 (4)	30 (9)	-	
Triple negative	19 (3)	19 (6)	-	

SMF: secondary myelofibrosis; PET MF: post essential thrombocythemia myelofibrosis; PPV MF: post polycythemia vera myelofibrosis; WBC: white blood cell count; Hb: hemoglobin level; PLT: platelet count.
*palpable from the left costal margin
**Karyotype was available in 377 patients

Table 2. Incidence of events during the follow-up of 781 patients with secondary myelofibrosis.

Incidence /100 patients-year (95% CI)	**PET MF** (n = 399)	**PPV MF** (n = 384)	***P* value**
Thrombosis	2.2 (1.6-3.2)	3.2 (2.3-4.4)	.1
Blast phase	2.5 (1.8-3.5)	1.9 (1.2-2.7)	.2
Mortality	6.5 (5.3-7.9)	8.4 (6.9-10)	.8

Table 3. Results of the multivariable analysis to define predictors of inferior survival in 685 molecularly annotated SMF patients

Covariates	HR	95% CI	P value	Risk coefficient Beta	Points assigned in the MYSEC-PM
Age at diagnosis of SMF	1.07	1.05-1.09	<.0001	0.068	0.15
Hemoglobin < 11 g/dL	2.3	1.6-3.3	<.0001	0.8	2
Platelet < 150 $x10^9$/L	1.7	1.2-2.5	.006	0.5	1
Circulating blast cells ≥ 3%	2.9	1.8-4.8	<.0001	1.1	2
CALR-unmutated genotype	2.6	1.2-5.3	.001	0.9	2
Constitutional symptoms	1.5	1.0-2.0	.03	0.4	1

HR: Hazard Ratio; CI: confidence interval

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