VOLUME 36 · NUMBER 4 · FEBRUARY 1, 2018

JOURNAL OF CLINICAL ONCOLOGY

RAPID COMMUNICATION

MIPSS70: Mutation-Enhanced International Prognostic Score System for Transplantation-Age Patients With Primary Myelofibrosis

Paola Guglielmelli, Terra L. Lasho, Giada Rotunno, Mythri Mudireddy, Carmela Mannarelli, Maura Nicolosi, Annalisa Pacilli, Animesh Pardanani, Elisa Rumi, Vittorio Rosti, Curtis A. Hanson, Francesco Mannelli, Rhett P. Ketterling, Naseema Gangat, Alessandro Rambaldi, Francesco Passamonti, Giovanni Barosi, Tiziano Barbui, Mario Cazzola, Alessandro M. Vannucchi, and Ayalew Tefferi

Author affiliations and support information (if applicable) appear at the end of this article.

Published at jco.org on December 9, 2017.

Processed as a Rapid Communication manuscript.

A.M.V and A.T. contributed equally to this work.

Corresponding author: Alessandro M. Vannucchi, MD, CRIMM, AOU Careggi, Università di Firenze, Viale Pieraccini, 6. 50134 Firenze, Italy; e-mail: amvannucchi@unifi.it.

0732-183X/18/3604w-310w/$20.00

ABSTRACT

Purpose

To develop a prognostic system for transplantation-age patients with primary myelofibrosis (PMF) that integrates clinical, cytogenetic, and mutation data.

Patients and Methods

The study included 805 patients with PMF age ≤ 70 years recruited from multiple Italian centers and the Mayo Clinic (Rochester, MN), forming two independent learning and validation cohorts. A Cox multivariable model was used to select from among a list of 22 variables those that were predictive of overall survival (OS). Integrated clinical and genetic prognostic models with (MIPSS70-plus) or without (MIPSS70) cytogenetic information were developed.

Results

Multivariable analysis identified the following as significant risk factors for OS: hemoglobin < 100 g/L, leukocytes > 25 × 10^9/L, platelets < 100 × 10^9/L, circulating blasts ≥ 2%, bone marrow fibrosis grade ≥ 2, constitutional symptoms, absence of *CALR* type-1 mutation, presence of high–molecular risk mutation (ie, *ASXL1*, *EZH2*, *SRSF2*, *IDH1/2*), and presence of two or more high–molecular risk mutations. By assigning hazard ratio (HR)–weighted points to these variables, three risk categories were delineated for the MIPSS70 model; 5-year OS was 95% in low-risk, 70% in intermediate-risk, and 29% in high-risk categories, corresponding to median OS of 27.7 years (95% CI, 22 to 34 years), 7.1 years (95% CI, 6.2 to 8.1 years), and 2.3 years (95% CI, 1.9 to 2.7 years), respectively. In the MIPSS70-plus model, which included cytogenetic information, four risk categories were delineated, with 5-year OS of 91% in low-risk, 66% in intermediate-risk (HR, 3.2; 95% CI, 1.9 to 5.2), 42% in high-risk (HR, 6.4; 95% CI, 4.1 to 10.0), and 7% very high–risk categories (HR, 17.0; 95% CI, 9.8 to 29.2). Both models remained effective after inclusion of older patients in the analysis.

Conclusion

MIPSS70 and MIPSS70-plus provide complementary systems of risk stratification for transplantation-age patients with PMF and integrate prognostically relevant clinical, cytogenetic, and mutation data.

J Clin Oncol 36:310-318. © 2017 by American Society of Clinical Oncology

INTRODUCTION

Primary myelofibrosis (PMF) is a myeloproliferative neoplasm characterized by hematopoietic stem-cell–derived clonal myeloproliferation that is often associated with bone marrow fibrosis.[1] Patients with PMF are at risk for premature death, and their quality of life is compromised by severe anemia, marked splenomegaly, profound constitutional symptoms, and cachexia.[2] Causes of death include leukemic transformation, disease progression without acute transformation, thrombosis, infections, bleeding, and complications of portal hypertension.[3]

There are currently three clinically derived prognostic models in PMF, including the International Prognostic Scoring System (IPSS),[4] the Dynamic IPSS (DIPSS),[5] and DIPSS-plus.[6] These models use five variables that independently predict inferior survival: age > 65 years, hemoglobin < 100 g/L, leukocyte count > 25 × 10^9/L,

ASSOCIATED CONTENT

Appendix
DOI: https://doi.org/10.1200/JCO.2017.76.4886

DOI: https://doi.org/10.1200/JCO.2017.76.4886

circulating blasts ≥ 1%, and constitutional symptoms. DIPSS-plus considers three additional variables, including RBC transfusion need, platelet count $< 100 \times 10^9$/L, and unfavorable karyotype.[7,8] In a Mayo Clinic study of 1,000 consecutive patients with PMF, DIPSS-plus was effective in identifying patient groups with median survival of 1.7 (high risk), 4.7 (intermediate-2 risk), 8.1 (intermediate-1 risk), and 19.2 years (low risk).[9]

In approximately 90% of patients, PMF is associated with one of three mutually exclusive driver mutations, including Janus kinase 2 (*JAK2*), calreticulin (*CALR*), and myeloproliferative leukemia virus oncogene (*MPL*).[10,11] Among these, *JAK2* has an estimated incidence of 65%, followed by *CALR* at 20% to 25% and *MPL* at 5% to 10%. In addition to these driver mutations, > 80% of patients with PMF harbor other DNA variants in myeloid genes, including *ASXL1*, *TET2*, *EZH2*, *SRSF2*, *DNMT3A*, *U2AF1*, and *IDH1*/*IDH2*, often in multiple combinations.[12] In addition to their presumed pathogenetic relevance, driver and other mutations in PMF have recently been shown to influence overall survival (OS) and leukemia-free survival (LFS), independent of IPSS and DIPSS-plus.[10,12-14] Current evidence supports prognostic distinction based on the presence or absence of type 1–like *CALR* mutations,[15,16] whereas *ASXL1*, *SRSF2*, *EZH2*, and *IDH1*/*IDH2* mutations are considered as high–molecular risk (HMR) mutations, the prognostic relevance of which is further amplified by the number of such mutations in an individual patient.[10,12,17]

Treatment of PMF includes supportive care, use of JAK2 inhibitors and other drugs, surgical removal or involved-field irradiation of the spleen, and allogeneic stem-cell transplantation (alloSCT).[2] These treatment measures, with the exception of alloSCT, are mostly palliative and unlikely to modify the natural history of the disease.[18] Unfortunately, alloSCT carries a substantial risk of treatment-related mortality and morbidity, which underscores the need for reliable prognostic models that facilitate treatment decision making and justify the risk involved in alloSCT in otherwise transplantation-eligible patients.[19] Accordingly, current treatment recommendations favor alloSCT for DIPSS/DIPSS-plus high- or intermediate-2–risk disease, whereas a more conservative treatment approach might be considered for lower-risk disease.[2,20]

In this multicenter study with training and validation cohorts of patients with PMF, we integrated clinical data with molecular and cytogenetic information and also accounted for bone marrow (BM) fibrosis grade.[11,21,22] The main objective was to develop new prognostic models, specifically directed toward transplantation-age patients, operationally defined as age ≤ 70 years, in line with current practice guidelines.

PATIENTS AND METHODS

Patient Cohorts and Clinical Procedures

Our study included two independent cohorts of patients with PMF, diagnosed according to the 2016 WHO criteria[23]; 676 patients were recruited from multiple Italian institutions associated with the Associazione Italiana per la Ricerca sul Cancro Gruppo Italiano Malattie Mieloproliferative project,[24] and 413 were from the Mayo Clinic (Rochester, MN). Samples for cytogenetic analysis and sequencing were collected within 2 years of diagnosis in the Italian cohort and at time of referral in the Mayo cohort. The study was approved by local institutional review boards and performed in accordance with the Declaration of Helsinki.

Mutational and Cytogenetic Analyses

Mutation analysis was performed on DNA from peripheral blood or BM cells. *JAK2V* 617F and *MPL* W515 mutations were detected by real-time polymerase chain reaction or high-resolution melting analysis.[10] *CALR* mutations were identified by capillary electrophoresis and bi-directional sequencing and classified as type 1– or type 2–like.[19,25] Next-generation sequencing[26] was used to detect mutations in selected myeloid genes, including *EZH2*, *ASXL1*, *IDH1*/*IDH2*, and *SRSF2*,[10] previously shown to be prognostically informative in PMF; an HMR category was defined by the presence of one or more of these mutations[10] (additional information is provided in the Appendix, online only). Cytogenetic analysis and reporting were performed according to the International System for Human Cytogenetic Nomenclature criteria[27] using standardized techniques.[28,29]

Statistical Analysis

For developing the MIPSS70 model, the Italian cohort was used as the learning set, because it contained substantially more patients with full molecular information; conversely, the Mayo cohort was used as the learning set for developing the MIPSS70-plus model, because of its greater number of patients with cytogenetic information. In each instance, the learning set was used to develop a prognostic scoring system, and the validation cohort was used to assess the prognostic ability of the new model. A Cox proportional hazards model with a stepwise selection procedure was used to select covariates, based on their statistical significance ($P < .05$), from among a list of variables with previously recognized prognostic relevance, including those used in the IPSS and DIPSS-plus risk models and other recently identified molecular and BM morphologic traits. Cutoffs for continuous variables were established by using results of the likelihood ratio test from the Cox model. Significant covariates were confirmed by forward-selection and backward-elimination techniques. On the basis of the magnitude of the hazard ratios (HRs) obtained from multivariable analysis, a weighted score was assigned to each significant variable for OS in the learning cohort; assignment of scores for the two models was performed independently. A new prognostic scoring system was subsequently developed, based on preliminary analysis of survival data, that corresponded to the sum of risk points and their operational classification based on demonstration of significant differences in survival. Survival was calculated as the interval between diagnosis or referral and death or last follow-up; patients who underwent alloSCT were censored on the date of transplantation. The cumulative probability of OS and LFS were estimated using the Kaplan-Meier method. Differences in OS and LFS among the groups were compared by a log-rank test. The relative goodness of fit of a new score was measured with the Akaike information criterion (AIC)[30]; the lower the AIC value, the more accurate and informative the prognostic model is. AIC measure was used as a simple summary method and was not used during model development. Receiver operating characteristic curve analysis and the area under the curve were also used to evaluate the ability of a new score to predict outcome. All values with $P < .05$ were considered statistically significant.

RESULTS

Patients

The study included 676 patients from four Italian institutions and 413 patients from the Mayo Clinic (Appendix Table A1, online only); of these, 490 (72.5%) and 315 (76.3%) were age ≤ 70 years and were included in development of the models (Table 1). Patients with overt PMF, anemia, leukocytosis, thrombocytopenia,

Table 1. Clinical and Laboratory Characteristics in Training and Validation Cohorts of Patients With PMF Age ≤ 70 Years for MIPSS70 and MIPSS70-Plus Prognostic Scores

Characteristic	MIPSS70		MIPSS70-Plus	
	Training (Italian) (n = 490)	Validation (Mayo) (n = 211)	Training (Mayo) (n = 315)	Validation (Italian) (n = 261)
Follow-up, years				
Median	5.85	4.65	4.6	6.0
Range	0.13-30.8	0.05-23.5	0.05-30.8	0.1-30.8
Prefibrotic PMF diagnosis	225 (45.9)	25 (11.8)	24 (11.8)[a]	125 (47.9)
Male sex	284 (58.0)	129 (61.1)	200 (63.5)	157 (60.2)
Age, years				
Median	55.4	60	60	55.6
Range	14.0-70.0	26.0-70.0	22.0-70.0	18.5-69.8
Hemoglobin, g/L				
Median	120	110	108	123
Range	44-175	58-160	58-167	50-175
Hemoglobin < 100 g/L	110 (22.4)	77 (36.5)	126 (40.0)	44 (16.8)
Leukocytes, $\times 10^9$/L				
Median	8.5	8	8.9	9.4
Range	1.5-106.1	1.1-176	1.1-176	1.6-100.0
Leukocytes > 25 $\times 10^9$/L	29 (5.9)	29 (13.7)	45 (14.3)	18 (6.9)
Leukocytes < 4.0 $\times 10^9$/L	45 (9.2)	36 (17.1)	51 (16.2)	20 (7.7)
Platelets, $\times 10^9$/L				
Median	370	229	230	384
Range	19-3,279	14-1,493	12-2,282	22-1,563
Platelets < 100 $\times 10^9$/L	53 (10.8)	48 (22.7)	67 (21.3)	25 (9.6)
Circulating blasts ≥ 2%	69 (14.1)	61 (28.9)	98 (31.1)	34 (13.0)
BM fibrosis grade				
1	175 (35.7)	22 (10.4)	21 (10.3)	103 (39.5)
2/3	265 (54.1)	186 (88.2)	180 (88.2)[a]	136 (52.1)
Constitutional symptoms	129 (26.3)	70 (33.2)	103 (32.7)	65 (24.9)
Patients with cytogenetics	261 (53.3)	204 (96.7)	315 (100)	261 (100)
Abnormal cytogenetics	72 (27.6)	81 (39.7)	132 (41.9)	72 (27.6)
Unfavorable karyotype[b]	29 (11.1)	45 (22.1)	77 (24.4)	29 (11.1)
IPSS/DIPSS-plus category	IPSS	DIPSS-plus	DIPSS-plus	IPSS
Low	229 (46.7)	35 (16.6)	50 (15.9)	127 (48.7)
Intermediate-1	133 (27.1)	41 (19.1)	61 (19.4)	70 (26.8)
Intermediate-2	69 (14.1)	82 (38.9)	124 (39.3)	37 (14.2)
High	59 (12.1)	53 (25.1)	80 (25.4)	27 (10.3)
Driver mutation				
JAK2 V617F	286 (58.4)	108 (51.2)	159 (50.5)	150 (57.5)
CALR type 1	96 (19.6)	57 (27.0)	86 (27.3)	53 (20.3)
CALR type 2	30 (6.1)	9 (4.3)	14 (4.4)	18 (6.9)
MPL W515x[c]	26 (5.3)	11 (5.2)	17 (5.4)	13 (5.0)
Triple negative[d]	52 (10.6)	26 (12.3)	39 (12.4)	27 (10.3)
ASXL1 mutation	113 (23.1)	80 (37.9)	113 (35.9)	62 (23.8)
EZH2 mutation	31 (6.3)	10 (4.7)	11 (3.7)[e]	17 (6.5)
SRSF2 mutation	34 (6.9)	30 (14.2)	42 (13.3)	20 (7.7)
IDH1/2 mutation	13 (2.7)	8 (3.8)	13 (4.4)[f]	7 (2.7)
HMR[g]	151 (30.8)	86 (40.6)	126 (40.9)	83 (31.8)
≥ 2 HMR mutations[h]	39 (8.0)	20 (9.5)	25 (7.9)	22 (8.4)
Acute leukemia progression	63 (12.9)	30 (14.2)	41 (13.0)	26 (10.0)
Death	190 (38.8)	134 (63.5)	208 (66.0)	102 (39.1)

NOTE. Data are given as No. (%) except where otherwise indicated.

Abbreviations: BM, bone marrow; DIPSS-plus, Dynamic International Prognostic Scoring System plus; HMR, high molecular risk; IPSS, International Prognostic Scoring System; PMF, primary myelofibrosis.

[a]n = 204.

[b]Unfavorable karyotype indicates any abnormal karyotype other than normal karyotype or sole abnormalities of 20q−, 13q−, +9, chromosome 1 translocation/duplication, −Y, or sex chromosome abnormality other than −Y (Tefferi et al, manuscript submitted for publication; A. Tefferi, personal communication, October 2017).

[c]*MPL* W515x indicates any mutation occurring at *MPL* codon 515.

[d]Triple negative indicates patients who lacked driver mutation.

[e]n = 295.

[f]n = 298.

[g]HMR category indicates presence of a mutation in any of the following genes in a patient: *ASXL1*, *EZH2*, *SRSF2*, or *IDH1/2*.

[h]Indicates presence of two or more mutated genes among *ASXL1*, *EZH2*, *SRSF2*, and *IDH1/2* in a patient; two or more mutations in the same gene are counted as one.

circulating blasts, BM fibrosis grade 2/3, unfavorable cytogenetics, and DIPSS-plus intermediate-2– or high-risk score were enriched in the Mayo cohort, as expected in a representative population of patients seen at different times after diagnosis, as opposed to the Italian cohort, which included newly diagnosed patients.[9] This was also reflected by a higher rate of death in the Mayo cohort (64% *v* 40%).

A driver mutation was found in 89% and 87% of patients in the Italian and Mayo cohorts, respectively: *JAK2* V617F in 58% and 51%, *CALR* type 1 in 20% and 27%, *CALR* type 2 in 6% and 4%, *MPL* W515x in 5% and 5%, and triple negative in 11% and 12%. HMR mutations were present in 31% and 41% of the Italian and Mayo patients, and presence of two or more HMR mutations was documented in 8% and 9%, respectively (Table 1). Cytogenetic information was available in 53% and 97% of the Italian and Mayo patients; 28% and 40% had abnormal karyotype, and 11% and 22% had an unfavorable karyotype, respectively (Table 1).

Development and Validation of Mutation-Enhanced IPSS: MIPSS70

We applied a Cox proportional hazards model using the Italian cohort as the training cohort (n = 490) and the Mayo cohort as the validation cohort. The model included the following variables (Table 2): hemoglobin < 100 g/L, abnormal leukocyte count (either < 4 or > 25 × 10^9/L), platelet count < 100 × 10^9/L, circulating blast count ≥ 2%, BM fibrosis grade, constitutional symptoms, IPSS/DIPSS-plus category, driver mutations, absence of *CALR* type 1–like mutation, individual HMR mutations, HMR category, and two or more HMR mutations.

Table 2 summarizes the results of univariable and multivariable analyses in the training cohort. The multivariable model identified nine independent predictors of survival: hemoglobin < 100g/L, leukocyte count > 25 × 10^9/L, platelet count < 100 × 10^9/L, circulating blasts ≥ 2%, fibrosis grade ≥ 2, constitutional symptoms, absence of *CALR* type 1–like mutation, HMR category, and two or more HMR mutations. For assignment of individual scores, we divided the HR value of each variable by the median HR value of all variables (Table 2). Accordingly, a weighted score of 1 was assigned to anemia, circulating blasts, fibrosis grade, constitutional symptoms, absence of *CALR* type 1–like mutation, and HMR category (individual HR range, 1.72 to 2.18), whereas a score of 2 was assigned to leukocytosis, thrombocytopenia, and two or more HMR mutations (HR range, 3.16 to 3.95). The overall score ranged from 0 to 12, with increasing scores indicating higher risk. On this basis, we constructed a three-category MIPSS70 risk model: low, score of 0 to 1; intermediate, score of 2 to 4; high, score ≥ 5. The model was then applied to the validation cohort (n = 211; Table 1). The HR for death in the validation cohort, using the low-risk category as reference, was 4.4 (95% CI, 1.8 to 11.1) for the intermediate-risk and 9.9 (95% CI, 3.9 to 24.7) for the high-risk categories (Table 3). The 5-year OS in the validation cohort was 96% in low-risk, 67% in intermediate-risk, and 34% in high-risk patients (Table 3; Figs 1A and 1B).

Table 2. Univariable and Multivariable OS Analyses in Patients With PMF Age ≤ 70 Years for Development of MIPSS70 (Italian cohort) and MIPSS70-Plus (Mayo cohort) Prognostic Scores

	MIPSS70		MIPSS70-Plus	
Variable	Univariable	Multivariable	Univariable	Multivariable
Hemoglobin < 100 g/L	3.32 (2.42 to 4.55); < .001	1.89 (1.32 to 2.71); < .001	1.78 (1.3 to 2.4); < .001	1.5 (1.1 to 2.0); .005
Leukocytes > 25 × 10^9/L	4.26 (1.26 to 11.46); < .001	3.8 (2.21 to 6.64); < .001	2.26 (1.6 to 3.2); < .001	
Leukocytes < 4.0 × 10^9/L	2.56 (1.65 to 3.96); < .001			
Platelets < 100 × 10^9/L	3.95 (2.66 to 5.86); < .001	3.16 (2.09 to 4.77); < .001	1.42 (1.0 to 2.0); .047	
Circulating blasts ≥ 2%	3.06 (2.23 to 4.21); < .001	1.72 (1.17 to 2.54); .006	2.08 (1.6 to 2.8); < .001	1.6 (1.2 to 2.3); .002
BM fibrosis grade		1.91 (1.34 to 2.71); < .001	2.12 (1.0 to 4.3); .04	
1	6.20 (1.94 to 19.85); .002			
≥ 2	12.9 (4.10 to 40.54); < .001			
Constitutional symptoms	2.78 (2.07 to 3.72); < .001	2.18 (1.57 to 3.03); < .001	2.56 (1.9 to 3.4); < .001	1.86 (1.4 to 2.5); < .001
CALR type 1 like				
Present	Reference	1.89 (1.21 to 2.96); .005	2.2 (1.5 to 3.2); < .001	2.4 (1.7 to 3.5); < .001
Absent	1.80 (1.04 to 3.05); .033			
Driver mutation				
CALR type 1 like	Reference			
CALR type 2	2.09 (1.10 to 3.95); .024			
JAK2 V617F/*MPL* W515x*	1.98 (1.31 to 2.98); .001			
Triple negative†	4.26 (2.51 to 7.25); < .001			
ASXL1 mutation	2.40 (1.80 to 3.21); < .001			
EZH2 mutation	1.88 (1.13 to 3.15); .016			
SRSF2 mutation	3.40 (2.22 to 5.20); < .001			
IDH1/2 mutation	4.51 (2.29 to 8.90); < .001			
HMR‡	2.46 (1.99 to 3.51); < .001	1.77 (1.26 to 2.49); .004	2.0 (1.5 to 2.7); < .001	1.8 (1.3 to 2.5); < .001
≥ 2 HMR mutations§	5.20 (3.45 to 7.83); < .001	3.95 (2.43 to 6.40); < .001	3.2 (2.0 to 5.1); < .001	2.4 (1.4 to 4.0); < .001
Unfavorable karyotype‖	ND	ND	2.66 (1.9 to 3.6); < .001	3.1 (2.3 to 4.3); < .001

NOTE. Data are given as HR (95% CI); *P*.
Abbreviations: BM, bone marrow; DIPSS-plus, Dynamic International Prognostic Scoring System plus; HMR, high molecular risk; HR, hazard ratio; IPSS, International Prognostic Scoring System; ND, not determined; OS, overall survival; PMF, primary myelofibrosis.
**MPL* W515x indicates any mutation occurring at *MPL* codon 515.
†Triple negative indicates patients who lacked driver mutation.
‡HMR category indicates presence of a mutation in any of the following genes in a patient: *ASXL1*, *EZH2*, *SRSF2*, or *IDH1/2*.
§Indicates presence of two or more mutated genes among *ASXL1*, *EZH2*, *SRSF2*, and *IDH1/2* in a patient; two or more mutations in the same gene are counted as one.
‖Unfavorable karyotype indicates any abnormal karyotype other than normal karyotype or sole abnormalities of 20q−, 13q−, +9, chromosome 1 translocation/duplication, −Y, or sex chromosome abnormality other than −Y (Tefferi et al, manuscript submitted for publication; A. Tefferi, personal communication, October 2017).

Table 3. OS by MIPSS70 and MIPSS70-Plus Prognostic Scores in Training and Validation Cohorts

Category (score range)	Training Cohort				Validation Cohort			
	No. (%) of Patients	Median (range) OS (years)	HR (95% CI)	*P*	No. (%) of Patients	Median (range) OS (years)	HR (95% CI)	*P*
MIPSS70	Italian cohort				Mayo cohort			
Low (0-1)	238 (48.6)	27.7 (21.7-33.7)	1.00		27 (12.8)	Not reached	1.00	
Intermediate (2-4)	198 (40.4)	7.1 (6.2-8.1)	5.5 (3.8 to 8.0)	< .001	105 (49.8)	6.3 (0.1-23.5)	4.4 (1.8 to 11.1)	< .001
High (≥ 5)	54 (11.0)	2.3 (1.9-2.7)	16.0 (10.2 to 25.1)	< .001	79 (37.4)	3.1 (0.05-14.6)	9.9 (3.9 to 24.7)	< .001
MIPSS70-plus	Mayo cohort				Italian cohort			
Low (0-2)	86 (27.3)	20.0 (1.0-23.5)	1.00		25 (9.6)	Not reached	1.00	
Intermediate (3)	63 (20.0)	6.3 (0.6-30.9)	3.2 (1.9 to 5.2)	< .001	108 (41.4)	24.2 (12.3-36.1)	1.8 (0.9 to 5.1)	.303
High (4-6)	127 (40.3)	3.9 (0.05-17.1)	6.4 (4.1 to 10.0)	< .001	79 (30.3)	10.4 (7.1-13.6)	4.8 (1.7 to 13.8)	.004
Very high (≥ 7)	39 (12.4)	1.7 (0.14-7.7)	17.0 (9.8 to 29.2)	< .001	49 (18.7)	3.9 (0.7-7.1)	11.7 (4.1 to 33.7)	< .001

Abbreviations: HR, hazard ratio; OS, overall survival.

Development and Validation of the Cytogenetic-Enhanced MIPSS70 Scoring System: MIPSS70-Plus

To appreciate the value of adding cytogenetics to the MIPSS70 system, we applied a Cox proportional hazards model among patients for whom both molecular and cytogenetic information were available; the Mayo cohort was used as the training cohort (n = 315) and the Italian cohort as the validation cohort. The model started by considering the same variables of MIPSS70 and included the presence or absence of unfavorable karyotype.

Table 2 summarizes the results of univariable and multivariable analyses in the training cohort. The multivariable model identified seven independent predictors of survival: hemoglobin < 100 g/L, circulating blasts ≥ 2%, constitutional symptoms, absence of *CALR* type 1–like mutation, HMR category, two or more HMR mutations, and unfavorable karyotype. For assignment of individual scores, the HR of each variable was rounded to the lower integer value (Table 2); therefore, a score of 1 was assigned to hemoglobin < 100 g/L, circulating blasts ≥ 2%, constitutional symptoms, and HMR category (HR range, 1.5 to 1.8); a score of 2 was assigned to absence of type 1–like *CALR* mutations and presence of two or more HMR mutations (HR, 2.4 for both); and a score of 3 was assigned to unfavorable karyotype (HR, 3.1); the overall score ranged from 0 to 12. On this basis, we constructed a four-category MIPSS70-plus risk model: low, score of 0 to 2; intermediate, score of 3; high, score of 4 to 6; and very high, score ≥ 7. The score was then applied to the validation cohort (n = 261 patients). The HR for death in the validation cohort, using the low-risk category as reference, was 1.8 (95% CI, 0.9 to 5.1) for the intermediate-risk, 4.8 (95% CI, 1.7 to 13.8) for the high-risk, and 11.7 (95% CI, 4.1 to 33.7) for the very high–risk categories (Table 3). The 5-year OS in the validation cohort was 100% in low-risk, 90% in intermediate-risk, 76% in high-risk, and 46.5% in very high–risk patients (Table 3; Figs 1C and 1D).

All Age–Inclusive Application of Novel Prognostic Scoring Systems

Although the new scores were developed specifically in patients age ≤ 70 years for facilitating alloSCT decision making, we wanted to evaluate if they remained informative after inclusion of older patients (Appendix Table A1). Using the MIPSS70 system in the Italian cohort, the HR for death was 4.2 (95% CI, 3.8 to 8.0) for the intermediate-risk and 12.2 (95% CI, 8.7 to 17.2) for the high-risk categories (Appendix Table A2, online only). The 5-year OS was 91.0% in low-risk, 55.9% in intermediate-risk, and 22.9% in high-risk patients (Appendix Fig A1A, online only). Using the MIPSS70-plus system in the Mayo cohort, the HR for death was 2.7 (95% CI, 1.8 to 4.1) for the intermediate-risk, 5.6 (95% CI, 3.9 to 8.2) for the high-risk, and 14.4 (95% CI, 9.1 to 22.7) for the very high–risk categories (Appendix Table A2). The 5-year OS was 85% in low-risk, 63% in intermediate-risk, 33% in high-risk, and 5% in very high–risk patients (Appendix Fig A1C). The scores were then successfully applied to each other cohort (Appendix Figs A1B and A1D; Appendix Table A2).

Comparisons of MIPSS70 With IPSS and MIPSS70-Plus With DIPSS-Plus

On the basis of receiver operating characteristic curve analysis, the area under the curve was 0.760 for MIPSS70 and 0.710 for IPSS, whereas the AIC was 2976.19 and 3401.41, respectively, supporting the performance of the new score. A similar analysis for MIPSS70-plus was not attempted, because of the different criteria used to designate unfavorable karyotype in DIPSS-plus and MIPSS70-plus. Regardless, we produced a cross table illustrating distribution of patients in the new scoring systems (rows in Fig 2) compared with their IPSS/DIPSS-plus risk categorization (colors within each row). Figure 2 illustrates significant risk redistributions when using MIPSS70/MIPSS70-plus across IPSS/DIPSS-plus risk categories. With regard to MIPSS70, we found that the score had overall good agreement for the low-risk category; 77% of patients in the MIPSS70 low-risk category were represented by IPSS low-risk patients (Fig 2A). Conversely, only 54% of patients in the MIPSS70 high-risk category were patients similarly considered at high risk in the IPSS system, whereas 46% of the patients had been upgraded from lower IPSS risk categories. The median OS of the latter patients was 2.3 to 3.7 years, indicating that they were appropriately identified by MIPSS70 as being at high risk of early death. Finally, the MIPSS70 intermediate-risk group was largely represented (65%) by patients of the intermediate-1 or intermediate-2 IPSS category, with 22% and 14% deriving from low- and high-risk IPSS categories, respectively; the median survival of patients in the latter two IPSS categories ranged from 5.2 to 6.4 years; therefore, they had been appropriately included in the

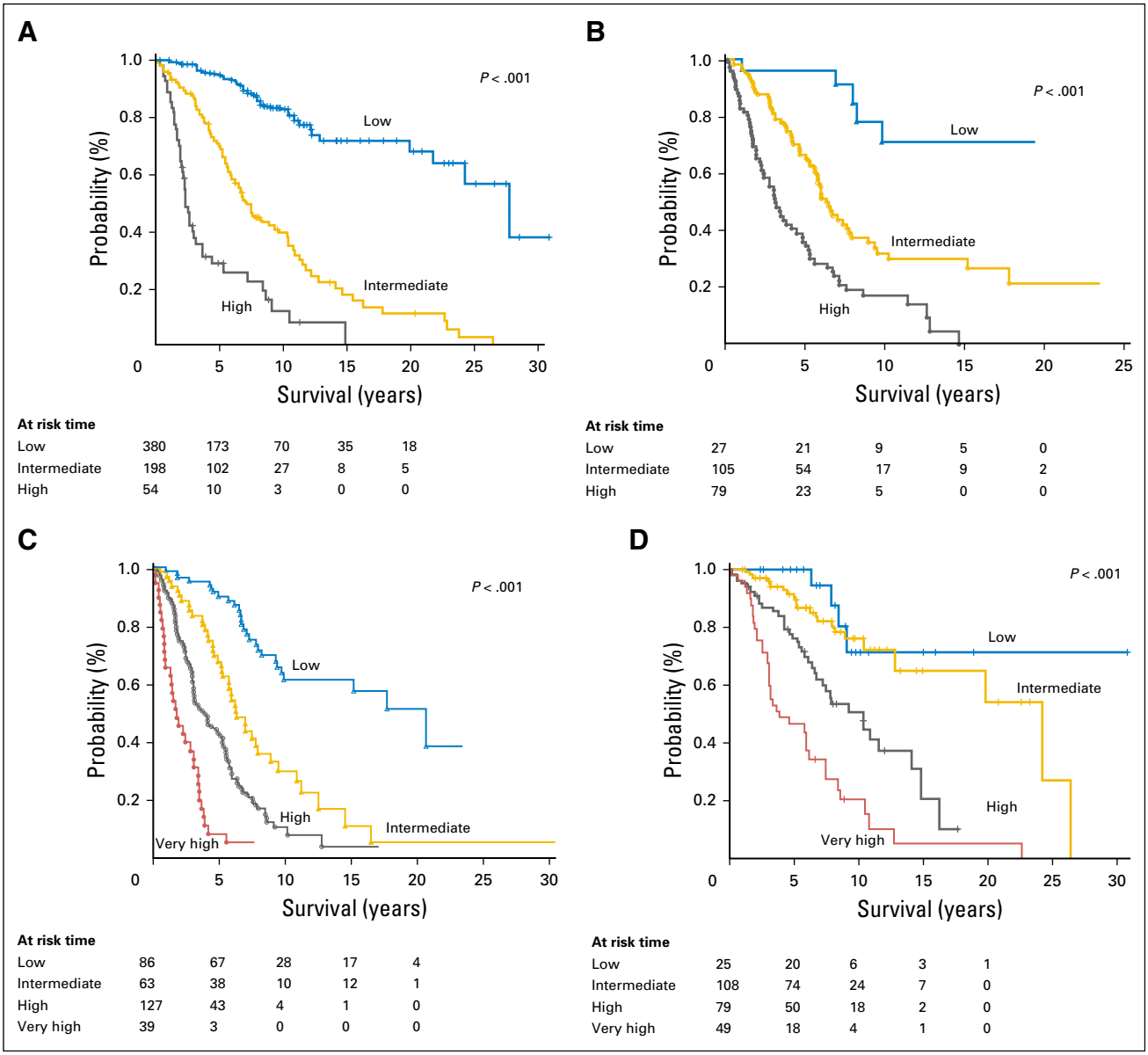

Fig 1. Overall survival (OS) in (A) training and (B) validation cohorts by the MIPSS70 prognostic scoring system risk classification. OS in (C) training and (D) validation cohorts by the MIPSS70-plus prognostic scoring system risk classification. Table 3 lists details. DIPSS-plus, Dynamic International Prognostic Scoring Systemplus; IPSS, International Prognostic Scoring System.

MIPSS70 intermediate category. Regarding MIPSS70-plus, the higher categories (high and very high) included 10.2% of patients originally classified as low or intermediate DIPSS-plus risk; conversely, 17 patients (19.8%) included in intermediate-2 DIPSS-plus group were downgraded to the lowest risk categories of MIPSS70-plus (Fig 2B).

Effect on LFS

Both models were also prognostic of LFS (Appendix Table A3, online only; Appendix Fig A2, online only). Because of the inclusion of cytogenetic information, MIPSS70-plus seemed to have the best performance in identifying a very high–risk category of patients age ≤ 70 years, 23% of whom developed acute leukemia (HR, 13.3; 95% CI, 4.7 to 37.4) as compared with 17.3% in high-risk (HR, 4.5; 95% CI, 2.0 to 10.3), 1.6% in intermediate-risk, and 11% in low-risk groups (Appendix Table A3; Appendix Fig A2C).

DISCUSSION

On the basis of consensus statements and guidelines, alloSCT, the only curative option in PMF, may be proposed when the expected survival of patients with conventional therapy is < 5 years.[20,31] In a retrospective comparative analysis of patients treated with drugs or alloSCT, the latter proved superior only in DIPSS intermediate-2 and high-risk patients, whose projected median survival was 4.5 and 2 years, respectively.[32] Careful evaluation of the intrinsic transplantation-related risk by specific scores like the Sorror index

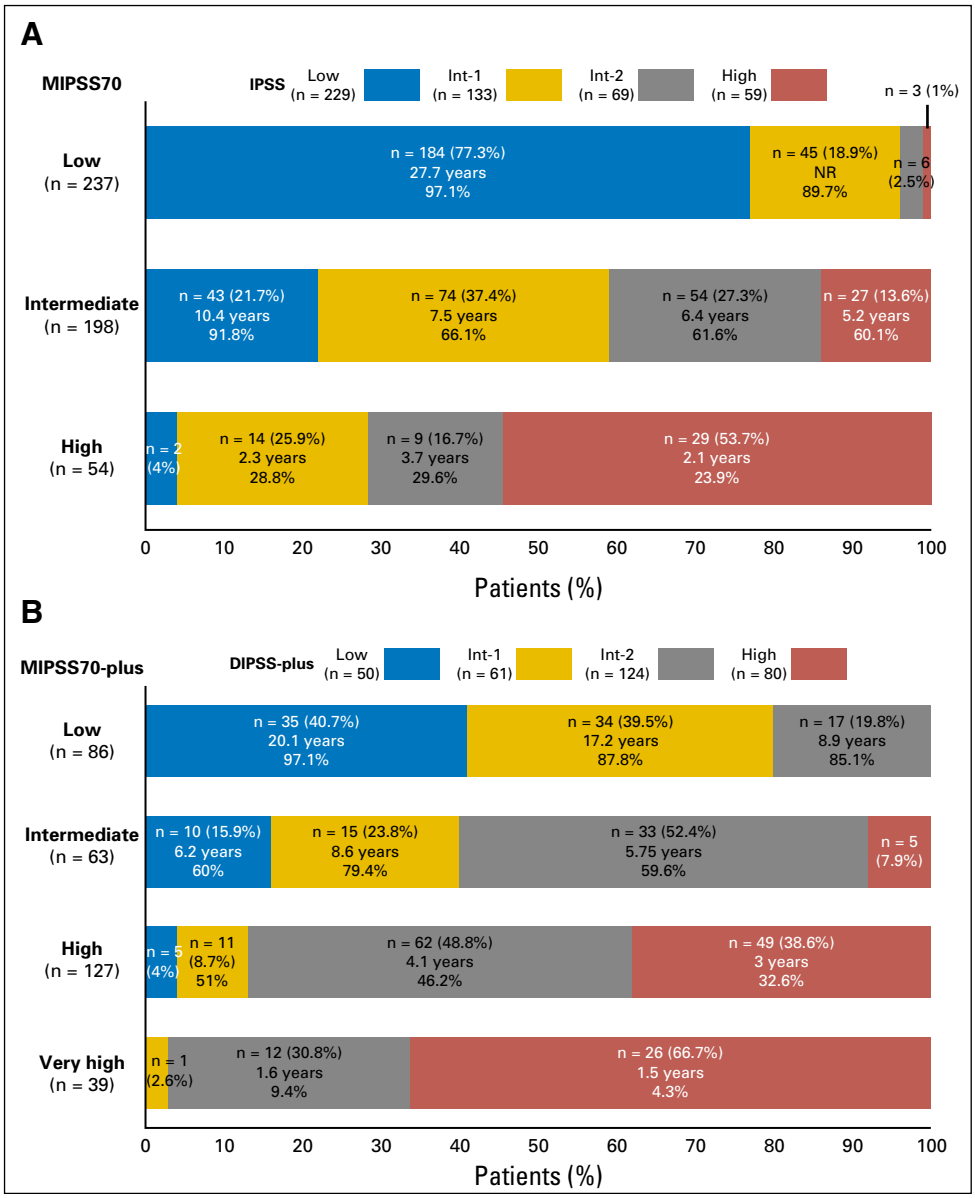

Fig 2. Categorization of patients according to (A) MIPSS70 and (B) MIPSS70-plus prognostic score versus the International Prognostic Scoring System (IPSS) and Dynamic IPSS-plus, respectively. Colored bars represent the IPSS/DIPSS-plus risk stratification (*x*-axis) in the context of the stratification based on the new scoring systems (represented by the rows). Shown is the number of patients for each IPSS/DIPSS-plus category within the new scoring system category, together with median overall survival (years) and 5-year survival (%). Survival data were omitted for groups with < 10 patients. Int, intermediate. DIPSS-plus, Dynamic International Prognostic Scoring System-plus; IPSS, International Prognostic Scoring System.

is strictly required in this fragile and usually advanced-age population before a decision to proceed with alloSCT is finally taken. With the aim of facilitating alloSCT decision making in patients with PMF, we developed two complementary novel scoring systems that integrate clinical, cytogenetic, and mutation data, specifically developed for patients age ≤ 70 years. We show here that the new systems accurately stratified patients based on their projected survival and might therefore improve counseling compared with previous ones, as well as facilitate design of clinical trials using the transplantation procedure.

The MIPSS70 score considered conventional clinically derived risk factors of the IPSS/DIPSS systems and was enriched by including recently discovered genetic risk factors and the WHO-recognized morphologic feature of prefibrotic PMF. In addition, MIPSS70-plus incorporated information from the recently revised cytogenetic risk stratification, allowing a more refined designation of unfavorable karyotype (Tefferi et al, manuscript submitted for publication). The primary objective in developing MIPSS70 and MIPSSS70-plus was to improve our ability to select patients for alloSCT procedure; however, both scores also identified different prognostic categories, irrespective of age, in 1,089 patients with PMF, indicating that they may be applied to any patient with PMF and be particularly suitable for including homogeneous groups of patients in intervention studies, especially when the primary end point is survival. To facilitate the use of the model, an interactive web application (available online at http://mipss70score.it) has been developed and is freely available.

The three-tiered MIPSS70 score reliably identified high-risk patients with a median OS of 2.3 years (95% CI, 1.9 to 2.7 years) and a risk of death of 81% at 5 years, which would justify the upfront use of alloSCT. Conversely, the long estimated survival in low-risk patients (27.7 years) favors deferring the particular treatment modality and also raises concerns about their inclusion in clinical trials. The risk involved in alloSCT might also not be

justified upfront in MIPSS70 intermediate-risk patients who have an estimated median survival of 7.1 years (95% CI, 6.2 to 8.1 years); close monitoring of clinical course and/or conventional or experimental treatment might be preferable in such patients. Although MIPSS70 was not developed as a dynamic system, it was nevertheless validated by an appropriate external cohort, the risk variables of which were dynamically collected at any time during the disease course.

The presence of HMR mutations does not necessarily coincide with the occurrence of unfavorable karyotype, and both variables have previously been shown to carry IPSS/DIPSS-independent and interindependent prognostic relevance, which was exploited in developing MIPSS70-plus. The latter complemented MIPSS70 by identifying patients at very high risk for both premature death and leukemic transformation. Such information is crucial for timing of alloSCT, and the comprehensive data on genetic risk factors provide additional assurance in reiterating the favorable outlook in low-risk patients. Finally, it should be noted that prospective controlled studies are required to clarify the role of alloSCT in rescuing patients with high-risk disease and that indication for alloSCT does not necessarily imply value.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at jco.org.

AUTHOR CONTRIBUTIONS

Conception and design: Paola Guglielmelli, Alessandro M. Vannucchi, Ayalew Tefferi
Financial support: Paola Guglielmelli, Mario Cazzola, Alessandro M. Vannucchi, Ayalew Tefferi
Provision of study materials or patients: Paola Guglielmelli, Animesh Pardanani, Mario Cazzola, Alessandro M. Vannucchi, Ayalew Tefferi
Collection and assembly of data: All authors
Data analysis and interpretation: Paola Guglielmelli, Terra L. Lasho, Giada Rotunno, Mythri Mudireddy, Carmela Mannarelli, Maura Nicolosi, Annalisa Pacilli, Curtis A. Hanson, Rhett P. Ketterling, Naseema Gangat, Mario Cazzola, Alessandro M. Vannucchi, Ayalew Tefferi
Manuscript writing: All authors
Final approval of manuscript: All authors
Accountable for all aspects of the work: All authors

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Affiliations

Paola Guglielmelli, **Giada Rotunno**, **Carmela Mannarelli**, **Annalisa Pacilli**, **Francesco Mannelli**, and **Alessandro M. Vannucchi**, University of Florence, Azienda Ospedaliero Universitaria Careggi, Florence; **Elisa Rumi**, **Vittorio Rosti**, **Giovanni Barosi**, and **Mario Cazzola**, Fondazione Istituto di Ricovero e Cura a Carattere Scientifico Policlinico San Matteo; **Elisa Rumi** and **Mario Cazzola**, University of Pavia, Pavia; **Alessandro Rambaldi**, University of Milan, Milan; **Alessandro Rambaldi** and **Tiziano Barbui**, Azienda Socio sanitaria Territoriale Papa Giovanni XXIII, Bergamo; **Francesco Passamonti**, University of Insubria, Varese, Italy; and **Terra L. Lasho**, **Mythri Mudireddy**, **Maura Nicolosi**, **Animesh Pardanani**, **Curtis A. Hanson**, **Rhett P. Ketterling**, **Naseema Gangat**, and **Ayalew Tefferi**, Mayo Clinic, Rochester, MN.

Support

Supported by a grant from Associazione Italiana per la Ricerca sul Cancro (AIRC; Milan, Italy), by the special program Molecular Clinical Oncology 5×1000 to the AIRC Gruppo Italiano Malattie Mieloproliferative (project 1005), by Progetto Ministero della Salute grant No. GR-2011-02352109 (P.G.), and by a grant from the Henry J. Predolin Foundation (Madison, WI) to the Mayo Clinic.

Prior Presentation

Presented at the 59th Annual Meeting of the American Society of Hematology, Atlanta, GA, December 9-12, 2017.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

MIPSS70: Mutation-Enhanced International Prognostic Score System for Transplantation-Age Patients With Primary Myelofibrosis

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Paola Guglielmelli
No relationship to disclose

Terra L. Lasho
No relationship to disclose

Giada Rotunno
No relationship to disclose

Mythri Mudireddy
No relationship to disclose

Carmela Mannarelli
No relationship to disclose

Maura Nicolosi
No relationship to disclose

Annalisa Pacilli
No relationship to disclose

Animesh Pardanani
No relationship to disclose

Elisa Rumi
No relationship to disclose

Vittorio Rosti
No relationship to disclose

Curtis A. Hanson
No relationship to disclose

Francesco Mannelli
No relationship to disclose

Rhett P. Ketterling
No relationship to disclose

Naseema Gangat
No relationship to disclose

Alessandro Rambaldi
No relationship to disclose

Francesco Passamonti
No relationship to disclose

Giovanni Barosi
No relationship to disclose

Tiziano Barbui
No relationship to disclose

Mario Cazzola
No relationship to disclose

Alessandro M. Vannucchi
No relationship to disclose

Ayalew Tefferi
No relationship to disclose

Appendix

The genomic DNA was extracted from density gradient–purified granulocytes of peripheral blood or mononuclear bone marrow cells. The *JAK2* V617F and *MPL* W515L/K mutations were assessed by real-time quantitative polymerase chain reaction (RTQ-PCR) only (*JAK*2) and by both RTQ-PCR and high-resolution melting analysis followed by bidirectional Sanger sequencing for *MPL*, as previous reported.[5,6] Mutations in exon 9 of *CALR* were assessed by capillary electrophoresis followed by bidirectional sequencing as described previously.[18] Capillary electrophoresis was performed using the ABI Prism 310 Genetic Analyzer (Applied Biosystems, Forest City, CA) followed by fragment analysis on GeneMapper software (version 4.1; Applied Biosystems). For fragment analysis, PCR was carried out with a 6-FAM–labeled forward primer. All samples with an additional peak to the wild-type one were further analyzed by Sanger sequencing. The level of detection was $< 0.1\%$ for *JAK2* V617F mutations and 1% for *MPL* W515 mutations using RTQ-PCR and HRMA and 1% by electrophoresis for *CALR* mutations.

Next-generation sequencing analysis was performed for the Associazione Italiana per la Ricerca sul Cancro Gruppo Italiano Malattie Mieloproliferative cohort. For target deep sequencing on Ion Torrent Personal Genome Machine (PGM) platform (Life Technologies, Carlsbad, CA), high-quality DNA was used to prepare genomic DNA libraries using Ion AmpliSeq Library Kit 2.0 (Life Technologies) and the Ion Xpress barcode adapters (Life Technologies). The libraries were purified with Agentcourt AMPure XP (Beckman Coulter, Brea, CA) and quantified with Ion Library Quantitation Kit (Life Technologies) on StepOne Plus system (Applied Biosystems). All these steps were carried out as per manufacturer instructions. *EZH2*, *ASXL1*, *IDH1*, *IDH2*, and *SRSF2* sequences were amplified using an Ion AmpliSeq Custom Panel (Life Technologies) that had been designed using the AmpliSeq Designer Tool 2.2.1 (Life Technologies). The primers for the customized panel were designed to cover coding exons of *EZH2* and *ASXL1* genes or hotspot regions of *IDH1*, *IDH2*, and *SRSF2* genes; in total, 36 amplicons were generated in two multiplex PCR reactions. All amplicons were subsequently amplified by emulsion PCR using Ion PGM Template OT2 (Life Technologies) and enriched using Ion PGM Enrichment Beads (Life Technologies). Sequencing was finally performed on PGM using Ion PGM Sequencing 200 Kit (version 2; Life Technologies) on an Ion 316 chip (version 1; Life Technologies). The BAM binary format sequence data raw reads went through adapter trimming, removal of reads shorter than 20 bp and of exact duplicates, and quality trimming. For all samples, the Ion Torrent PGM raw reads were aligned against human reference genome 19 using NextGENe software (version 2.3.1; SoftGenetics, State College, PA). Each variant within the exonic regions and indel detection of targeted genes was confirmed by conventional sequencing (Sanger methodology). Functionally annotated variants were filtered based on the information retrieved from public databases (Single Nucleotide Polymorphism database [dbSNP], 1000 Genomes Project) and an internal control group of 100 controls. The potential pathogenetic role of filtered variants was assessed using available tools (SIFT, Polyphen, Catalogue of Somatic Mutations in Cancer [COSMIC]).

Molecular studies were performed for the Mayo cohort. DNA from either bone marrow or granulocytes was prepared, and 500 ng was submitted for high-resolution target capture sequencing. For the latter, paired-end indexed libraries were prepared from individual patient DNA in the Mayo Genomic Sequencing Core Laboratory using the NEBNext Ultra Library prep protocol on the Agilent Bravo liquid handler (NEB, Ipswich, MA/Agilent Technologies, Santa Clara, CA). Capture libraries were assembled according to Nimblegen standard library protocol (Roche Nimblegen, Basel, Switzerland). A panel including the regions of 27 heme-related genes was selected for custom target capture using Agilent SureSelect Target Enrichment Kit (Agilent Technologies). Capture libraries were pooled at equimolar concentrations and loaded onto paired-end flow cells at concentrations of 7 to 8 pM to generate cluster densities of 600,000 to 800,000/mm^2 following the Illumina (San Diego, CA) standard protocol using the Illumina cBot and HiSeq Paired End Cluster Kit (version 3; Illumina) in batches of 48 samples per lane. The flow cells were sequenced as 101×2 paired-end reads on an Illumina HiSEquation 2000 using TruSeq SBS Sequencing Kit (version 3; Illumina) and HiSeq data collection software (version 2.0.12.0; Illumina). Base-calling was performed using Illumina's RTA version 1.17.21.3.

Genesifter software (PerkinElmer, Danvers, MA) was used to analyze targeted sequence data. Reads from the sequencing in fastq format were aligned using Burrows-Wheeler Aligner against the genomic reference sequence for *Homo sapiens* (Build 37.2; National Center for Biotechnology Information; http://www.ncbi.nlm.nih.gov/). An additional alignment postprocessing set of tools was then used to perform local realignment, duplicate marking, and score recalibration to generate a finale genomic aligned set of reads. Nucleotide variants were called using the Genome Analysis Toolkit (Broad Institute, Cambridge, MA), which identified single-nucleotide and small insertion/deletion events using default settings. Alamut Visual mutation analysis software (Interactive Biosoftware, Rouen, France) was used to help filter variations through public genomic databases. Variants were not further analyzed if they did not have a sequencing read depth of ≥ 50 reads and/or were present with $\geq 1\%$ minor allele frequency (MAF) in normal population via the National Center for Biotechnology Information dbSNP. Remaining variants were further filtered through the Wellcome Trust Sanger Institute COSMIC public database and characterized into three categories: VAR (variants not previously

associated with a hematologic malignancy [by COSMIC] and present with ≤ 1% MAF in dbSNP), HEME (variants previously associated with a hematologic malignancy [by COSMIC] and present with ≤ 1% MAF in dbSNP), and MUT (variants previously associated with a hematologic malignancy and also identified as being somatic [by COSMIC or previously by Mayo Clinic Research Laboratory (data not shown)] and present with ≤ 1% MAF in dbSNP).

Table A1. Clinical and Laboratory Characteristics in Learning and Validation Cohorts of Patients With PMF of All Ages for Both MIPSS70 and MIPSS70-Plus

	MIPSS70		MIPSS70-Plus	
Characteristic	Training Cohort (n = 676)	Validation Cohort (n = 275)	Learning Cohort (n = 413)	Validation Cohort (n = 347)
Follow-up, years				
Median	4.90	3.9	3.9	5.3
Range	0.13-30.8	0.04-23.5	0.04-30.9	0.14-30.8
Prefibrotic PMF diagnosis	286 (42.3)	35 (12.7)	34 (12.8)[a]	158 (45.5)
Male sex	414 (61.2)	168 (61.1)	263 (63.7)	221 (63.7)
Age, years				
Median	61.5	62	63	60.4
Range	14.0-90.3	26-87	22-87	18.5-88.7
Hemoglobin, g/L				
Median	118	106	104	120
Range	40-175	58-160	58-167	40-175
Hemoglobin < 100 g/L	185 (27.4)	115 (41.8)	191 (46.2)	73 (21.0)
Leukocytes, $\times 10^9$/L				
Median	8.7	8.6	9	9.6
Range	1.4-109.0	1.1-176	1-218.5	1.6-109.0
Leukocytes > 25 $\times 10^9$/L	52 (7.7)	36 (13.1)	61 (14.8)	32 (9.2)
Leukocytes < 4.0 $\times 10^9$/L	68 (10.1)	46 (16.7)	64 (15.5)	26 (7.5)
Platelets, $\times 10^9$/L				
Median	325	227	224	382
Range	19-3,279	14-1,493	11-2,466	22-1,563
Platelets < 100 $\times 10^9$/L	87 (12.9)	64 (23.3)	92 (22.3)	37 (10.7)
Circulating blasts ≥ 2%	98 (14.5)	79 (28.7)	130 (31.5)	50 (14.4)
BM fibrosis grade				
1	230 (34.0)	32 (11.6)	31 (11.7)	132 (38.0)
2/3	390 (57.7)	240 (87.3)	231 (87.2)[a]	189 (54.5)
Constitutional symptoms	187 (27.7)	90 (32.7)	142 (34.4)	90 (25.5)
Patients with cytogenetics	347 (51.3)	265 (96.4)	413 (100.0)	347 (100.0)
Abnormal cytogenetics	98 (28.2)	106 (40.0)	175 (42.4)	98 (28.2)
Unfavorable karyotype[b]	43 (12.4)	58 (21.9)	100 (24.2)	43 (12.4)
IPSS/DIPSS-plus category	IPSS	DIPSS-plus	DIPSS-plus	IPSS
Low	229 (33.9)	35 (12.7)	50 (12.1)	127 (36.6)
Intermediate-1	191 (28.3)	50 (18.2)	73 (17.7)	102 (29.4)
Intermediate-2	132 (19.5)	105 (38.2)	151 (36.6)	61 (17.6)
High	124 (18.3)	85 (30.9)	139 (33.6)	57 (16.4)
Driver mutation				
JAK2 V617F	416 (61.5)	150 (54.5)	224 (54.2)	209 (60.2)
CALR type 1	105 (15.5)	61 (22.2)	93 (22.5)	57 (16.4)
CALR type 2	34 (5.0)	11 (4.0)	18 (4.4)	20 (5.8)
MPL W515x[c]	39 (5.8)	16 (5.8)	25 (6.1)	17 (4.9)
Triple negative[d]	82 (12.1)	37 (13.5)	53 (12.8)	44 (12.7)
ASXL1 mutation	177 (26.2)	105 (38.2)	154 (37.3)	91 (26.2)
EZH2 mutation	57 (8.4)	13 (4.7)	15 (3.9)[e]	30 (8.6)
SRSF2 mutation	68 (10.1)	45 (16.4)	61 (14.8)	36 (10.4)
IDH1/2 mutation	19 (2.8)	12 (4.4)	18 (4.6)[f]	10 (2.9)
HMR[g]	246 (36.4)	110 (40.0)	171 (41.4)	128 (36.9)
≥ 2 HMR mutations[h]	67 (9.9)	30 (10.9)	35 (8.5)	36 (10.4)
Acute leukemia progression	80 (11.8)	37 (13.5)	52 (12.6)	36 (10.4)
Death	324 (47.9)	193 (70.2)	301 (72.9)	152 (43.8)

NOTE. Data are given as No. (%) except where otherwise indicated.
Abbreviations: BM, bone marrow; DIPSS-plus, Dynamic International Prognostic Scoring System plus; HMR, high molecular risk; IPSS, International Prognostic Scoring System; PMF, primary myelofibrosis.
[a]n = 265.
[b]Unfavorable karyotype indicates any abnormal karyotype other than normal karyotype or sole abnormalities of 20q−, 13q−, +9, chromosome 1 translocation/duplication, −Y, or sex chromosome abnormality other than −Y (Tefferi et al, manuscript submitted for publication; A. Tefferi, personal communication, October 2017).
[d]*MPL* W515x indicates any mutation occurring at *MPL* codon 515.
[e]Triple negative indicates patients who lacked driver mutation.
[f]n = 388.
[g]n = 390.
[h]HMR category indicates presence of a mutation in any of the following genes in a patient: *ASXL1*, *EZH2*, *SRSF2*, or *IDH1/2*.
[i]Indicates presence of two or more mutated genes among *ASXL1*, *EZH2*, *SRSF2*, and *IDH1/2* in a patient; two or more mutations in the same gene are counted as one.

Table A2. OS by MIPSS70 and MIPSS70-Plus Scores in Training and Validation Cohorts (all ages)

Category (score range)	Training Cohort				Validation Cohort			
	No. (%) of Patients	OS (years), Median (range)	HR (95% CI)	*P*	No. (%) of Patients	OS (years), Median (range)	HR (95% CI)	*P*
MIPSS70	Italian cohort				Mayo cohort			
Low (0-1)	284 (42.0)	27.7 (20.2-35.2)	1.00		30 (10.9)	Not reached	1.00	
Intermediate (2-4)	293 (43.3)	5.6 (4.9-6.3)	4.2 (3.8 to 8.0)	< .001	138 (50.2)	5.8 (0.1-23.5)	4.1 (1.9 to 8.9)	< .001
High (≥ 5)	99 (14.7)	2.1 (1.8-2.5)	12.2 (8.7 to 17.2)	< .001	107 (38.9)	2.9 (0.04-14.6)	8.5 (3.9 to 18.4)	< .001
MIPSS70-plus	Mayo cohort				Italian cohort			
Low (0-2)	99 (24.0)	15.2 (0.1-23.5)	1.00		25 (7.2)	Not reached	1.00	
Intermediate (3)	75 (18.1)	6.0 (0.6-30.9)	2.7 (1.8 to 4.1)	< .001	127 (36.6)	24.2 (12.3-36.1)	2.0 (0.7 to 5.8)	.189
High (4-6)	185 (44.8)	3.1 (0.05-17.1)	5.6 (3.9 to 8.2)	< .001	116 (33.4)	7.5 (5.3-9.7)	5.6 (2.0 to 15.9)	.001
Very high (≥ 7)	54 (13.1)	1.4 (0.04-7.7)	14.4 (9.1 to 22.7)	< .001	79 (22.8)	3.2 (3.0-3.4)	14.4 (5.1 to 40.5)	< .001

Abbreviations: DIPSS-plus, Dynamic International Prognostic Scoring Systemplus; HR, hazard ratio; IPSS, International Prognostic Scoring System; OS, overall survival.

Table A3. LFS by MIPSS70 and MIPSS70-Plus Scores in Training and Validation Cohorts

Category (score range)	Training Cohort				Validation Cohort			
	No. (%) of Patients	No. (%) of Events	HR (95% CI)	*P*	No. (%) of Patients	No. (%) of Events	HR (95% CI)	*P*
MIPSS70	Italian cohort				Mayo cohort			
Low (0-1)	238 (48.6)	14 (5.9)	1.00		27 (12.8)	3 (11.1)	1.00	
Intermediate (2-4)	198 (40.4)	34 (17.3)	5.1 (2.7 to 9.8)	< .001	105 (49.8)	15 (14.3)	1.9 (0.5 to 6.6)	.315
High (≥ 5)	54 (11.0)	15 (27.8)	17.4 (8.0 to 37.9)	< .001	79 (37.4)	12 (15.2)	3.1 (0.8 to 11.6)	.083
MIPSS70-plus	Mayo cohort				Italian cohort			
Low (0-2)	86 (27.3)	9 (10.5)	1.00		25 (9.6)	0	1.00	
Intermediate (3)	63 (20.0)	1 (1.6)	0.2 (0.0 to 2.0)	.191	108 (41.4)	8 (7.4)	2.2 (0.3 to 17.3)	.467
High (4-6)	127 (40.3)	22 (17.3)	4.5 (1.9 to 10.3)	< .001	79 (30.3)	9 (11.5)	3.7 (0.5 to 28.9)	.218
Very high (≥ 7)	39 (12.4)	9 (23.0)	13.3 (4.7 to 37.4)	< .001	49 (18.7)	9 (18.4)	8.6 (1.1 to 68.1)	.042

Abbreviations: DIPSS-plus, Dynamic International Prognostic Scoring Systemplus; HR, hazard ratio; IPSS, International Prognostic Scoring System; LFS, leukemia-free survival.

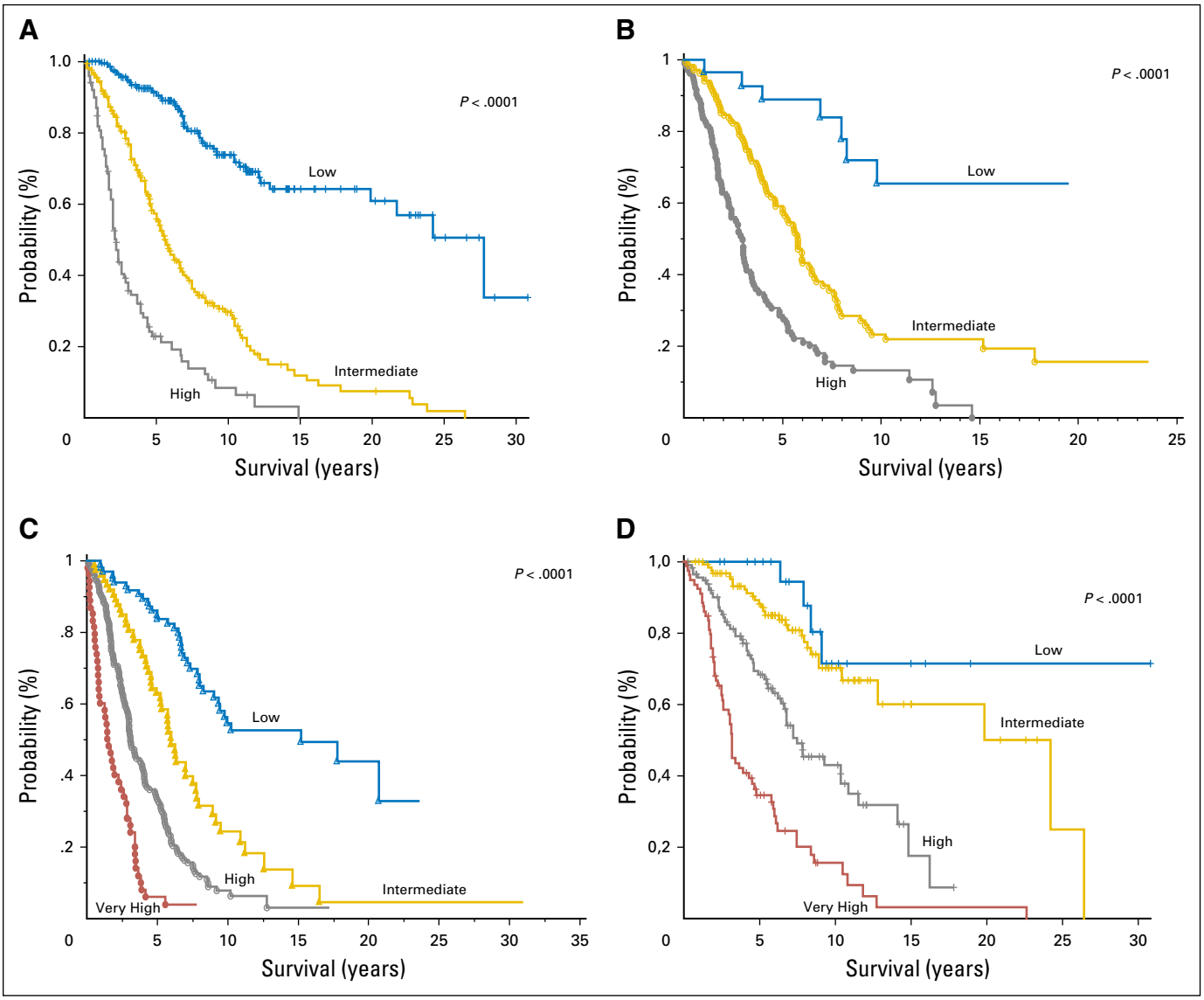

Fig A1. Overall survival (OS) in (A) learning and (B) validation cohorts by the MIPSS70 prognostic scoring system risk classification in all age–inclusive cohorts. OS in (C) learning and (D) validation cohorts by the MIPSS70-plus prognostic scoring system risk classification in all age–inclusive cohorts. Appendix Table A2 lists details.

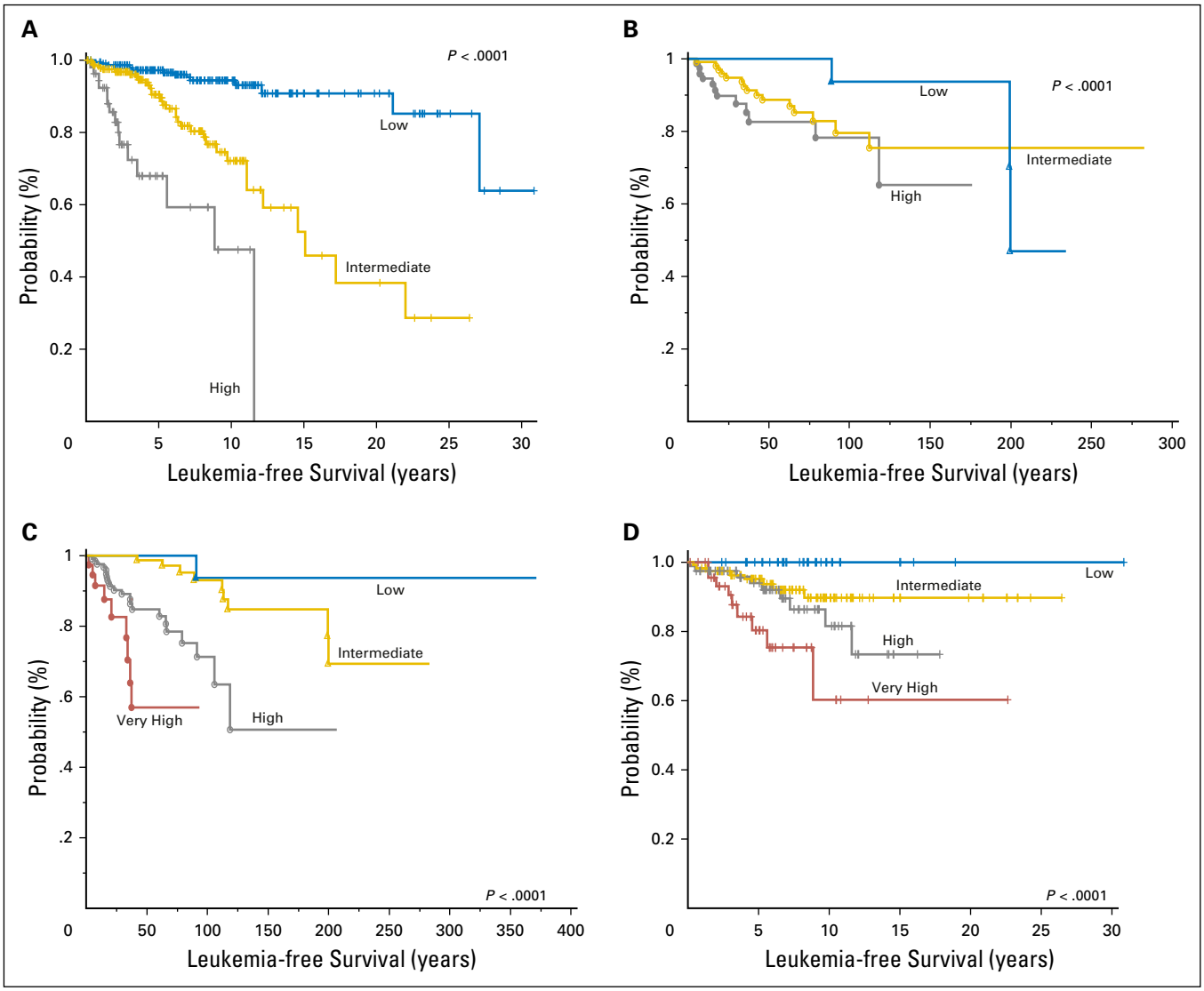

Fig A2. Leukemia-free survival (LFS) in (A) learning and (B) validation cohorts by the MIPSS70 prognostic scoring system risk classification. LFS in (C) learning and (D) validation cohorts by the MIPSS70-plus prognostic scoring system risk classification. Appendix Table A3 lists details.