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ORIGINAL ARTICLE

Mutations and prognosis in primary myelofibrosis

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Patient outcome in primary myelofibrosis (PMF) is significantly influenced by karyotype. We studied 879 PMF patients to determine the individual and combinatorial prognostic relevance of somatic mutations. Analysis was performed in 483 European patients and the seminal observations were validated in 396 Mayo Clinic patients. Samples from the European cohort, collected at time of diagnosis, were analyzed for mutations in *ASXL1*, *SRSF2*, *EZH2*, *TET2*, *DNMT3A*, *CBL*, *IDH1*, *IDH2*, *MPL* and *JAK2*. Of these, *ASXL1*, *SRSF2* and *EZH2* mutations inter-independently predicted shortened survival. However, only *ASXL1* mutations (HR: 2.02; $P<0.001$) remained significant in the context of the International Prognostic Scoring System (IPSS). These observations were validated in the Mayo Clinic cohort where mutation and survival analyses were performed from time of referral. *ASXL1*, *SRSF2* and *EZH2* mutations were independently associated with poor survival, but only *ASXL1* mutations held their prognostic relevance (HR: 1.4; $P=0.04$) independent of the Dynamic IPSS (DIPSS)-plus model, which incorporates cytogenetic risk. In the European cohort, leukemia-free survival was negatively affected by *IDH1/2*, *SRSF2* and *ASXL1* mutations and in the Mayo cohort by *IDH1* and *SRSF2* mutations. Mutational profiling for *ASXL1, EZH2, SRSF2* and *IDH* identifies PMF patients who are at risk for premature death or leukemic transformation.

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INTRODUCTION

Primary myelofibrosis (PMF) is a myeloproliferative neoplasm[1] whose cardinal features include extensive extramedullary hematopoiesis, associated with marked hepatosplenomegaly, anemia that is often transfusion-dependent and profound constitutional symptoms.[2] Median survival is estimated at <6 years with causes of death, including leukemic transformation, progressive cachexia, vascular events and infections.[3] At present, the only treatment modality with curative potential is allogeneic stem cell transplantation, but this particular treatment is still associated with a high rate of mortality and morbidity.[4] Other treatment options are primarily palliative and include splenectomy, involved field radiotherapy,[5] erythropoiesis stimulating agents, androgen preparations and thalidomide and its analogs to treat anemia, hydroxyurea and JAK inhibitors to treat splenomegaly or constitutional symptoms.[6–8] However, none of these drugs have been shown to either reverse bone marrow fibrosis or induce genetic remissions and their effect on survival is uncertain.

Physicians taking care of patients with PMF are often faced with therapeutic decisions that include the indication and timing of allogeneic stem cell transplantation or investigational drug therapy. Such decisions rely upon currently available prognostic scoring systems, recently developed by the International Working Group for myeloproliferative neoplasm Research and Treatment (IWG-MRT), including the International Prognostic Scoring System (IPSS),[3] which is applicable at time of diagnosis, and the dynamic IPSS (DIPSS) that can be applied at any time during the disease course.[9] Both IPSS and DIPSS use five adverse factors, including age >65 years, hemoglobin <10 g/dl, leukocyte count $>25\times10^9$/l, circulating blasts ⩾1% and presence of constitutional symptoms, in order to distinguish among low, intermediate-1, intermediate-2 and high-risk patients with respective median survivals of 11.3, 7.9, 4.0 and 2.3 years, per IPSS, or not reached, 14.2, 4.0 and 1.5 years, per DIPSS. DIPSS was recently modified into DIPSS-plus[10] by incorporating three additional DIPSS-independent risk factors: platelet count $<100\times10^9$/l,[11] red cell transfusion need[12,13] and unfavorable karyotype;[14,15] median survival for the low, intermediate-1, intermediate-2 and high-risk categories were 15.4, 6.5, 2.9 and 1.3 years. The current study seeks to improve upon these prognostic models by incorporating recently described molecular markers (somatic mutations), some of which have already been shown useful in the prognostic assessment of patients with acute myeloid leukemia (AML).[16]

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PATIENTS AND METHODS

Patients

This international collaborative study, approved by the individual center institutional review boards, included 879 patients with PMF diagnosed according to the 2008 WHO criteria;[1] patients with the 'prefibrotic' variant were excluded. The main objective was to evaluate the prognostic relevance of recently discovered mutations in PMF in the context of currently used prognostic models. In order to examine prognostic relevance of mutations detected at the time of initial diagnosis and further validate their value when encountered at time of tertiary center referral, we used two independent patient cohorts. Initial analysis was performed on 483 European patients studied within 1 year of diagnosis; the seminal observations were subsequently validated in an independent cohort of 396 patients studied at time of referral (including both newly and previously diagnosed patients) to the Mayo Clinic and receiving treatment according to current practice.[5]

Mutational analysis

For the European cohort, the Sanger technique was used to detect mutations across the entire coding region of *EZH2* and *TET2*, and regions previously described as mutational hotspots for *DNMT3A*, *CBL*, *ASXL1*, *IDH1*, *IDH2*, *SRSF2* and *MPL*; *JAK2*V617F was detected by real-time PCR(RT-PCR). Similar techniques were used for analyzing the Mayo samples for mutations found to be prognostically relevant in the European cohort: *ASXL1*, *SRSF2*, *EZH2*, *IDH1* and *IDH2* (see Supplementary Methods and Supplementary Table S1 for details).

Gene expression profiling

Gene expression profiling was performed on total cellular RNA isolated from $CD34^+$ cells of a subset of patients from the European cohort, randomly selected among those defined at 'mutationally high-risk' or 'mutationally low-risk' as described in Supplementary Methods.

Statistical analysis

The main end points were overall and leukemia-free survival. Actuarial survival curves were estimated by the method of Kaplan–Meier and compared by the log-rank test. For the European cohort, survival was measured from the date of diagnosis to the date of death or last follow-up and for the Mayo cohort from time of referral to date of death or last follow-up. Patients who underwent allogeneic stem cell transplantation were censored at the time of transplant. Multivariable analyses were conducted with the Cox proportional hazards regression model. The prognostic strength of mutations to predict transformation into AML was evaluated in the framework of competing risks; that is, the cumulative incidence of progression to AML was calculated by taking AML-unrelated death as competing risk.[17] Multivariable analyses of factors predicting progression to AML were also performed within the framework of competing risks by the sub-hazard regression method.[18] The mutual exclusivity of pairs of mutations was estimated with two-by-two contingencies tables and Fisher's exact test. Association between mutations and clinical and biological features was assessed by the χ^2 test. *P*-values <0.05 were considered significant.

RESULTS

Clinical presentation and disease course

Clinical and laboratory characteristics of all 879 study patients, stratified into European ($n=483$) and Mayo ($n=396$) cohorts, are outlined in Table 1. Median follow-up was 3.7 years (range 0–27.9) for the European and 3.4 years (range 0–26) for the Mayo cohorts. During follow-up, 157 (32.5%) deaths were recorded in the European cohort and 246 (62%) in the Mayo cohort; 75 (16%) and 46 (12%) leukemic transformations were documented in the European and Mayo cohort, respectively.

For the European cohort, median overall survival from time of diagnosis was 9.7 years (95% CI: 7.9–12.2) (Supplementary Figure S1A); the respective values for IPSS low, intermediate-1, intermediate-2 and high-risk disease were 22.8 years (95% CI: 12.2- not reached), 10.5 years (95% CI: 7.12–23.7), 6.2 years (95% CI: 5.2–9.5) and 2.5 years (95% CI: 1.7–2.8) ($P<0.0001$) (Supplementary Figure S1B). Unfavorable karyotype had an IPSS-independent detrimental effect on overall survival (HR: 6.46; 95% CI: 3.4–12.5; $P<0.001$) (Supplementary Figures S2A and B) and, in univariate analysis, on leukemia-free survival (HR: 3.45; 95% CI:

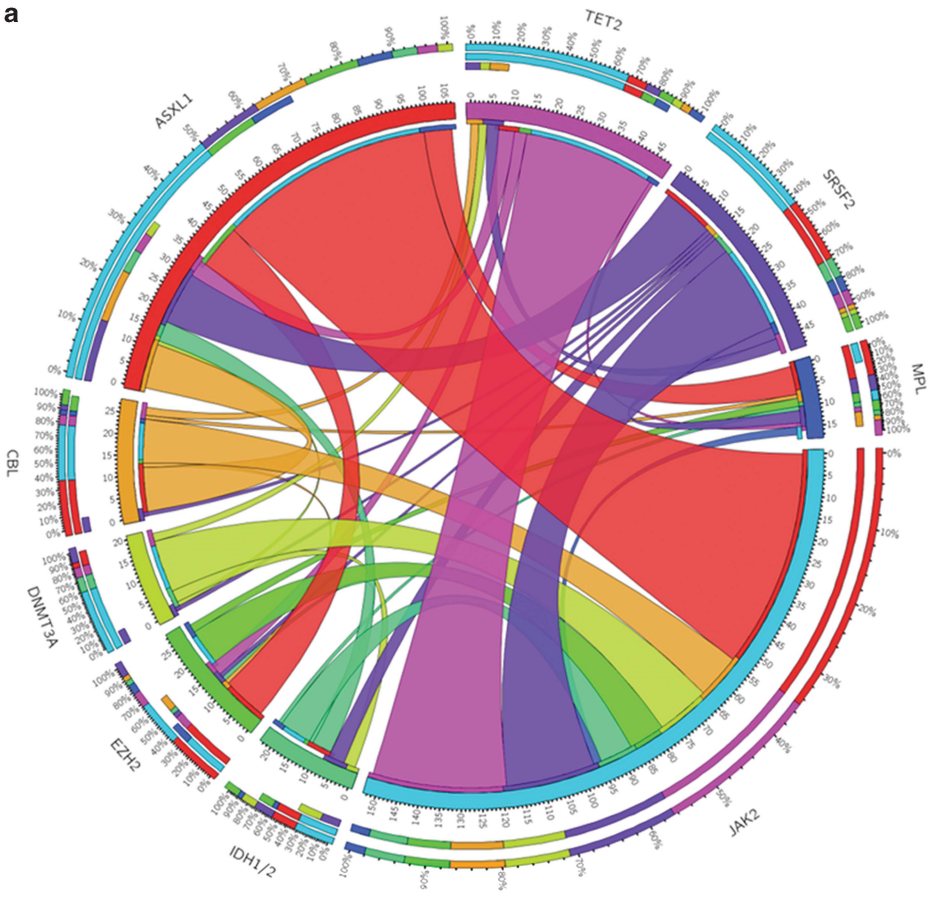
Table 1. Clinical and laboratory characteristics of 879 patients with PMF stratified into European ($n=483$) and Mayo Clinic ($n=396$) cohorts

Variables	*European cohort (at time of diagnosis)* (n = *483*)	*Mayo Clinic cohort (at time of referral)* (n = *396*)
Age in years; median (range)	61 (14–90)	63 (14–87)
Age >65 years; *n* (%)	188 (39%)	154 (39%)
Males (%)	296 (61%)	257 (65%)
Hemoglobin, g/dl; median (range)	11.4 (4.4–16.5)	10 (5.8–16.1)
Hemoglobin <10 g/dl; *n* (%)	135 (28%)	200 (51%)
Transfusion requiring; *n* (%)	NA	132 (33%)
Leukocytes, $\times 10^9$/l; median (range)	9 (1.4–106)	9 (1–176)
Leukocytes $>25\times10^9$/l; *n* (%)	38 (8%)	59 (15%)
Platelets, $\times 10^9$/l; median (range)	342 (7–3279)	228 (11–2466)
Platelets $<100\times10^9$/l; *n* (%)	57 (12%)	83 (21%)
Constitutional symptoms; *n* (%)	137 (28%)	139 (35%)
Circulating blasts ⩾1%; *n* (%)	80 (17%)	234 (59%)
Cytogenetic categories; *n* (%)	*'N' evaluable = 226*	*'N' evaluable = 393*
Abnormal	53 (24%)	154 (39%)
Unfavorable karyotype**	13 (6%)	40 (10%)
IPSS risk group; n (%)		
Low	166 (34%)	NA
Intermediate-1	146 (30%)	NA
Intermediate-2	104 (22%)	NA
High	67 (14%)	NA
DIPSS-plus risk group; n (%)		
Low	NA	49 (12%)
Intermediate- 1	NA	66 (17%)
Intermediate- 2	NA	150 (38%)
High	NA	131 (33%)
Palpable spleen; *n* (%)	355 (75%)	292 (74%)

Abbreviations: DIPSS-plus, dynamic international prognostic scoring system-plus[10]; IPSS, international prognostic scoring system.[3] Mutation analysis and survival calculations in the European cohort were performed from the time of diagnosis and in the Mayo cohort from the time of referral to the Mayo Clinic. **Unfavorable karyotype indicates any of the following: +8, –7/7q–, i(17q), inv(3), –5/5q, 12p–, or 11q23 rearrangements.

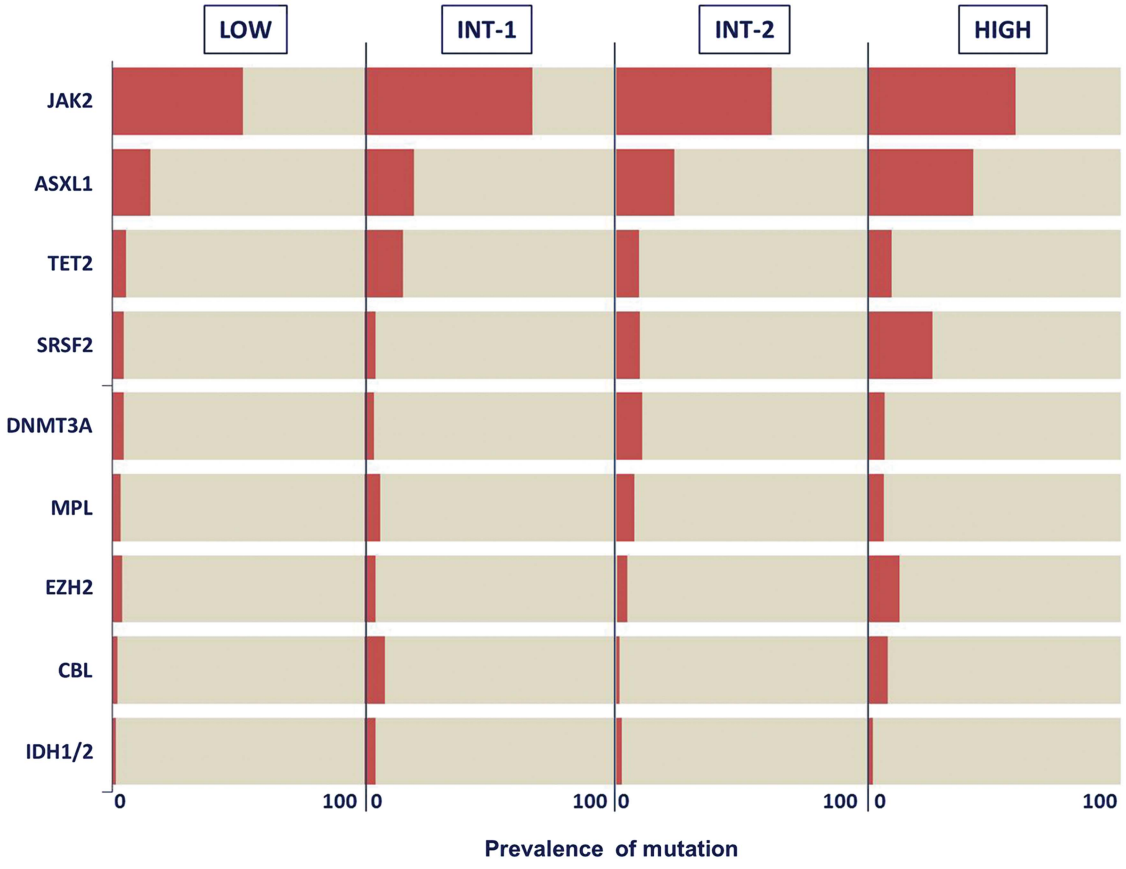
Figure 1. The frequency and the pair-wise co-occurrence of mutations in the 483 PMF patients included in the European cohort are represented by a Circos diagram in (**a**). Co-occurring mutations are indicated in the clockwise direction. In the Circos representation, the length of the arc corresponds to the frequency of mutation in the first gene (color coded) and the width of the ribbon corresponds to the frequency of patients who also had a mutation in the second gene. The frequency of mutations in this cohort is shown on the right side. (**b**) shows the prevalence proportion of individual mutations in the four IPSS risk categories.

a



Gene	Overall Frequency (%)
JAK2	59.2
ASXL1	21.7
TET2	9.7
SRSF2	8.5
DNMT3A	5.7
MPL	5.2
EZH2	5.1
CBL	4.4
IDH1/2	2.6

b



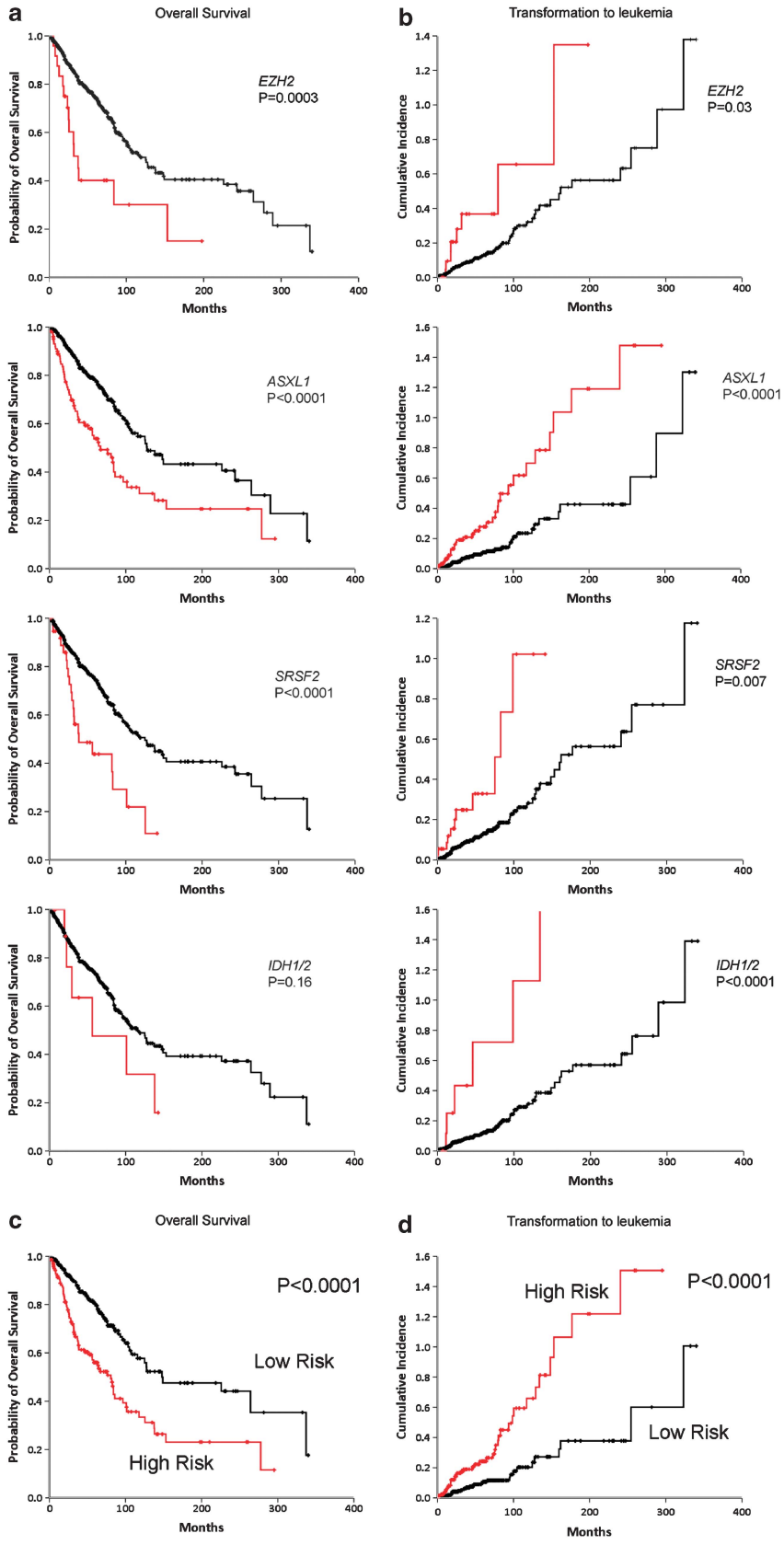


Figure 2. Kaplan–Meier estimates of overall survival in patients of the European series depending on the mutational status (wild-type or mutated) of *EZH2*, *ASXL1*, *SRSF2* and *IDH1* or *IDH2* mutation is presented in (**a**). The impact of mutations in *EZH2*, *ASXL1*, *SRSF2* and *IDH1* or *IDH2* on the cumulative risk of transformation to acute leukemia using a competitive risk analysis is shown in (**b**). The *P*-values reported in each panel refer to the comparison of mutated and wild-type subjects. A comparison of overall survival by Kaplan–Meier estimates (**c**) and the cumulative risk of transformation to acute leukemia (**d**) in patients considered at 'mutationally high-risk' (that is, those mutated at least in one of *ASXL1*, *EZH2*, *SRSF2*, *IDH1* or *IDH2*) versus 'mutationally low-risk' (that is, no mutation) is presented.

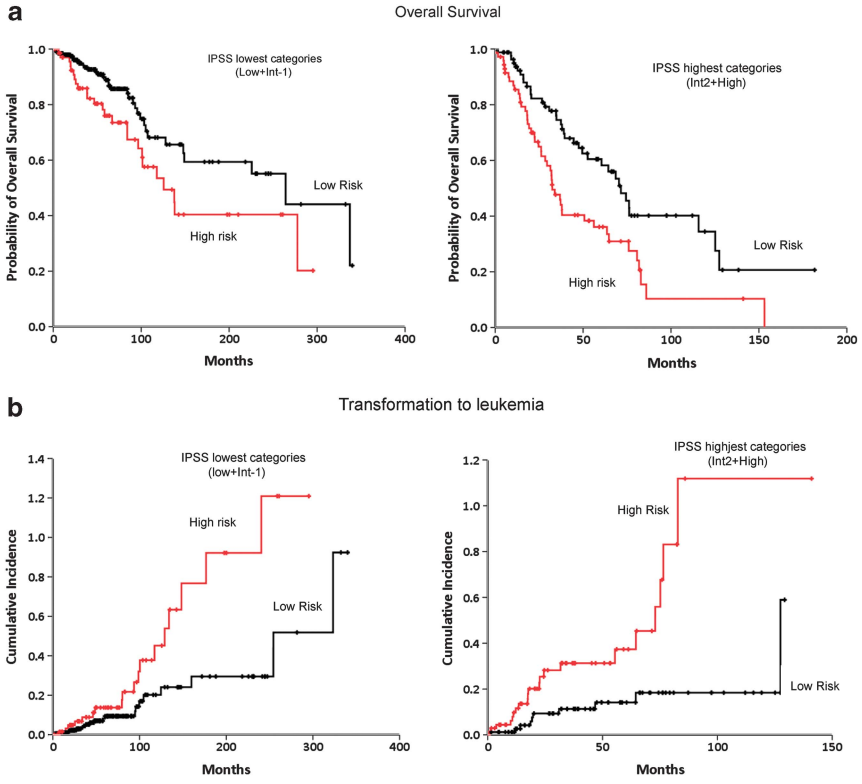


Figure 3. Kaplan–Meier estimates of overall survival in patients at 'mutationally high-risk' compared with 'mutationally low-risk' in the settings of IPSS categories; a lowest and a highest IPSS category were considered, the first including IPSS low plus Intermediate-1, the latter Intermediate-2 plus high-risk categories (**a**). In (**b**), the cumulative risk of transformation to acute leukemia in the same patient populations as in (**a**) is shown.

1.03–11.6; $P=0.033$) (Supplementary Figure S2C); the latter retained borderline significant in multivariable analysis (HR: 3.3; 95% CI: 0.98–11.3; $P=0.053$). For the Mayo cohort, median survival from time of referral was 5 years and significantly different among DIPSS-plus risk groups ($P<0.0001$); the median survivals for low, intermediate-1, intermediate-2 and high-risk groups were 18.7, 8.3, 5.2 and 2.3 years (Supplementary Figure S3).

Frequency and distribution of mutations

Among patients in the European cohort, 382 (79%) displayed at least one somatic mutation (see Supplementary Figure S4 for details); the frequency of individual mutations and their inter-relationships are shown in Figure 1a. *MPL* mutations were less frequent (0.7% versus 12%; $P<0.001$) in *JAK2*V617F-mutated patients; *IDH1* and *IDH2* mutations, which were mutually exclusive, were more frequent in *DNMT3A*-(27% versus 5%; $P=0.001$) or *SRSF2*-(36% versus 7%; $P<0.001$) mutated patients. *ASXL1* mutations were more frequent in *EZH2*-(11% versus 3%; $P<0.002$), *TET2*-(11% versus 4%; $P=0.043$), *DNMT3A*-(11% versus 2%; $P=0.022$), *CBL*-(11% versus 2%; $P<0.001$) and *SRSF2*-(13.5% versus 7%; $P=0.035$) mutated patients. Thirty-one patients (6.4%) displayed >2 mutations (Supplementary Table S2 and Supplementary Figure S5). As of their prognostic relevance in the European cohort (see below), mutation analyses in the Mayo cohort were focused on *ASXL1*, *SRSF2*, *EZH2*, *IDH1* and *IDH2*; the corresponding mutational frequencies were 31% (85 out of 279 studied cases), 15% (52 of 358), 6% (15 of 270), 3% (10 of 374) and 2% (7 of 374). Significant clustering was noted for *SRSF2* with both *IDH1* ($P=0.0002$) and *IDH2* ($P=0.0009$) mutations. One patient displayed four mutations (*ASXL1*, *SRSF2*, *EZH2* and *IDH2*) and four patients displayed three mutations (two displayed *ASXL1*, *SRSF2* and *EZH2* and two others displayed *ASXL1*, *SRSF2* and *IDH1* mutations).

Clinical and cytogenetic correlates of mutations

In the European cohort *ASXL1*, *SRSF2* and *EZH2* mutations were significantly ($P<0.05$) enriched in the IPSS high-risk group with mutational frequencies of 42%, 25% and 12%, respectively (Figure 1b). Also in the Mayo cohort *ASXL1* and *SRSF2* mutations were more enriched in the DIPSS-plus high-risk category (45%, $P=0.0002$ and 22%, $P=0.004$, respectively), with no difference for *EZH2* mutations.

In the European cohort, *JAK2*V617F mutation was associated with older age ($P=0.005$), higher hemoglobin level ($P=0.0001$) and increased leukocyte count ($P=0.001$); *EZH2* mutations with leukocytosis ($P=0.01$) and $\geqslant 1\%$ circulating blasts ($P=0.02$); *ASXL1* mutations with leukocytosis ($P<0.001$), $\geqslant 1\%$ circulating blasts ($P=0.001$), anemia ($P=0.01$), splenomegaly ($P=0.007$) and constitutional symptoms ($P<0.01$); *SRSF2* mutations with older age ($P<0.001$), leukocytosis ($P<0.01$), $\geqslant 1\%$ circulating blasts ($P=0.012$) and constitutional symptoms ($P=0.009$). There were no significant associations with cytogenetic risk groups.

In the Mayo cohort, *ASXL1* mutations clustered with older age ($P=0.01$), constitutional symptoms ($P<0.0001$), leukocytosis ($P=0.007$) and $\geqslant 1\%$ circulating blasts ($P=0.02$); *SRSF2* mutations with older age ($P=0.002$) and anemia/transfusion need ($P<0.05$); *EZH2* mutations with $\geqslant 1\%$ circulating blasts ($P=0.003$). Also in the Mayo cohort, *ASXL1* mutations were more likely to occur in the presence of normal karyotype (37% versus 19%; $P=0.003$) and were not different between favorable and unfavorable cytogenetic categories. *EZH2*, *SRSF2* and *IDH1/2* mutational frequencies were similar in patients with normal versus abnormal karyotype.

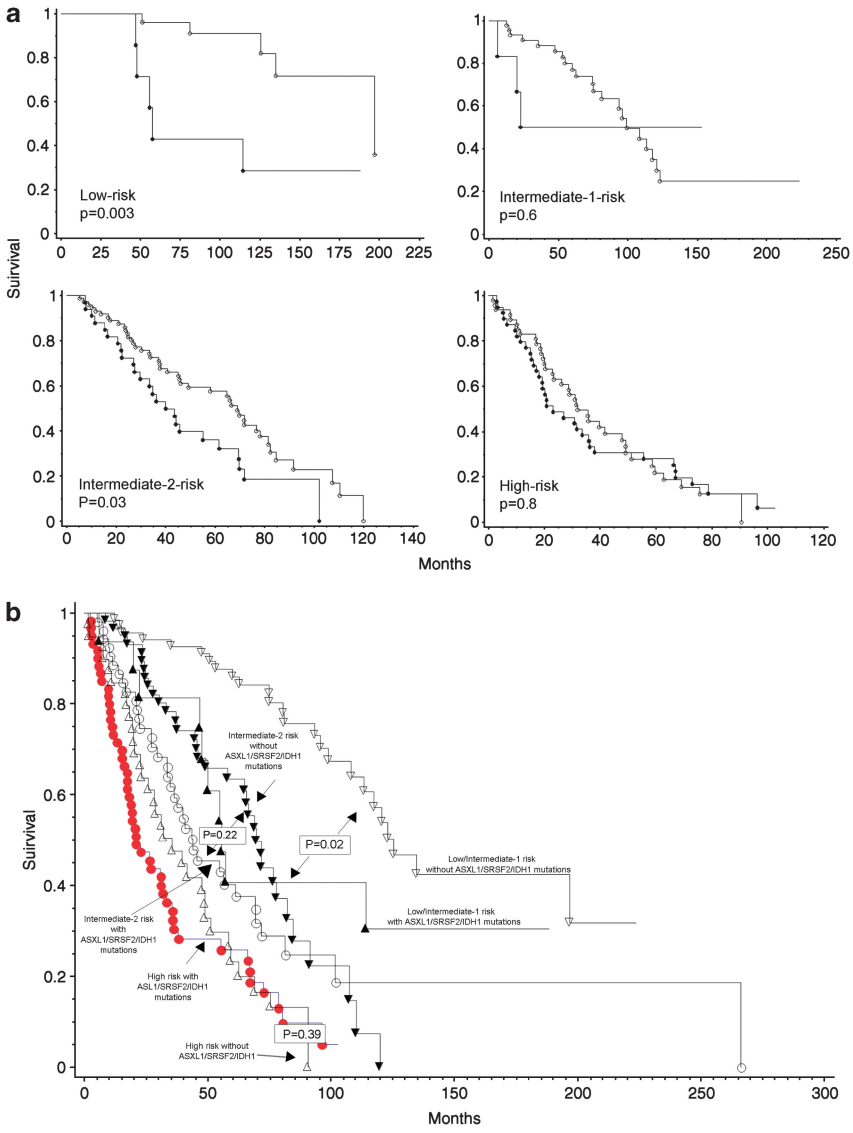


Figure 4. Three-tiered dynamic DIPSS-plus stratified survival data in 279 patients with PMF sub-stratified according to *ASXL1* mutation status (**a**) and according to *ASXL1/SRSF2/IDH1* mutation status (**b**).

Overall and leukemia-free survival correlates of mutations

European cohort. Univariate analysis disclosed significant correlations between shortened survival and *EZH2* ($P=0.0003$), *ASXL1* ($P<0.0001$) and *SRSF2* ($P<0.0001$) mutations (Figure 2a). A multivariable analysis that included all three mutations as covariates confirmed their inter-independent prognostic value: HR was 1.91 (95% CI: 1.1–3.36; $P=0.025$) for *EZH2*, 2.21 (95% CI: 1.57–3.11; $P<0.0001$) for *ASXL1* and 2.6 (95% CI: 1.63–41.6; $P<0.0001$) for *SRSF2* mutations. Risk of leukemia was significantly increased in *EZH2*-($P=0.03$), *ASXL1*-($P<0.0001$), *SRSF2*-($P=0.007$) and *IDH1*- or *IDH2*-($P<0.0001$) mutated patients (Figure 2b). In multivariable analysis that included the four mutations as covariates, *ASXL1* (HR: 2.5, 95% CI: 1.5–4.1; $P<0.0001$), *SRSF2* (HR: 2.73, 95% CI: 1.34–5.55; $P=0.005$) and *IDH1* or *IDH2* (HR: 2.66, 95% CI: 1.10–6.47; $P=0.03$), but not *EZH2* (HR: 1.98, 95% CI: 0.88–4.46), mutations remained significant.

As *EZH2*, *ASXL1* and *SRSF2* mutations predicted overall survival, while *ASXL1*, *SRSF2* and *IDH1* or *IDH2* predicted leukemic transformation, all independent of each other, we considered the four mutations (*ASXL1*, *EZH2*, *SRSF2* and *IDH1/2*) as being detrimental for disease outcome, in general, and accordingly classified the European patients into those who displayed at least one ('mutationally high-risk') or none ('mutationally low-risk') of the four mutations. Median survival was significantly shorter in the mutationally high-risk compared with the low-risk category (81 versus 148 months; $P<0.0001$) (Figure 2c). Multivariable analysis confirmed the independent prognostic value for survival of distinguishing mutationally low from high-risk groups in the context of IPSS ($P<0.0001$), cytogenetic risk stratification ($P<0.0001$) or both ($P=0.04$). However, when the four mutations were considered individually, only *ASXL1* mutations retained their significance in the context of IPSS (HR: 2.02; $P<0.001$).

Leukemia-free survival was significantly shorter in the mutationally high-risk group ($P<0.0001$) and the cumulative risk of leukemia using competitive risk analysis was significantly increased (HR: 2.96; 95% CI: 1.85–4.76; $P<0.0001$) compared with mutationally low-risk (Figure 2d); this was confirmed by multivariable analysis that included IPSS ($P<0.001$), unfavorable karyotype ($P<0.007$) or both ($P=0.024$) (Supplementary Table S3). The significant difference in overall and leukemia-free survival between the mutationally high- and low-risk groups was apparent when lower-risk categories (low plus intermediate-1 risk patients)

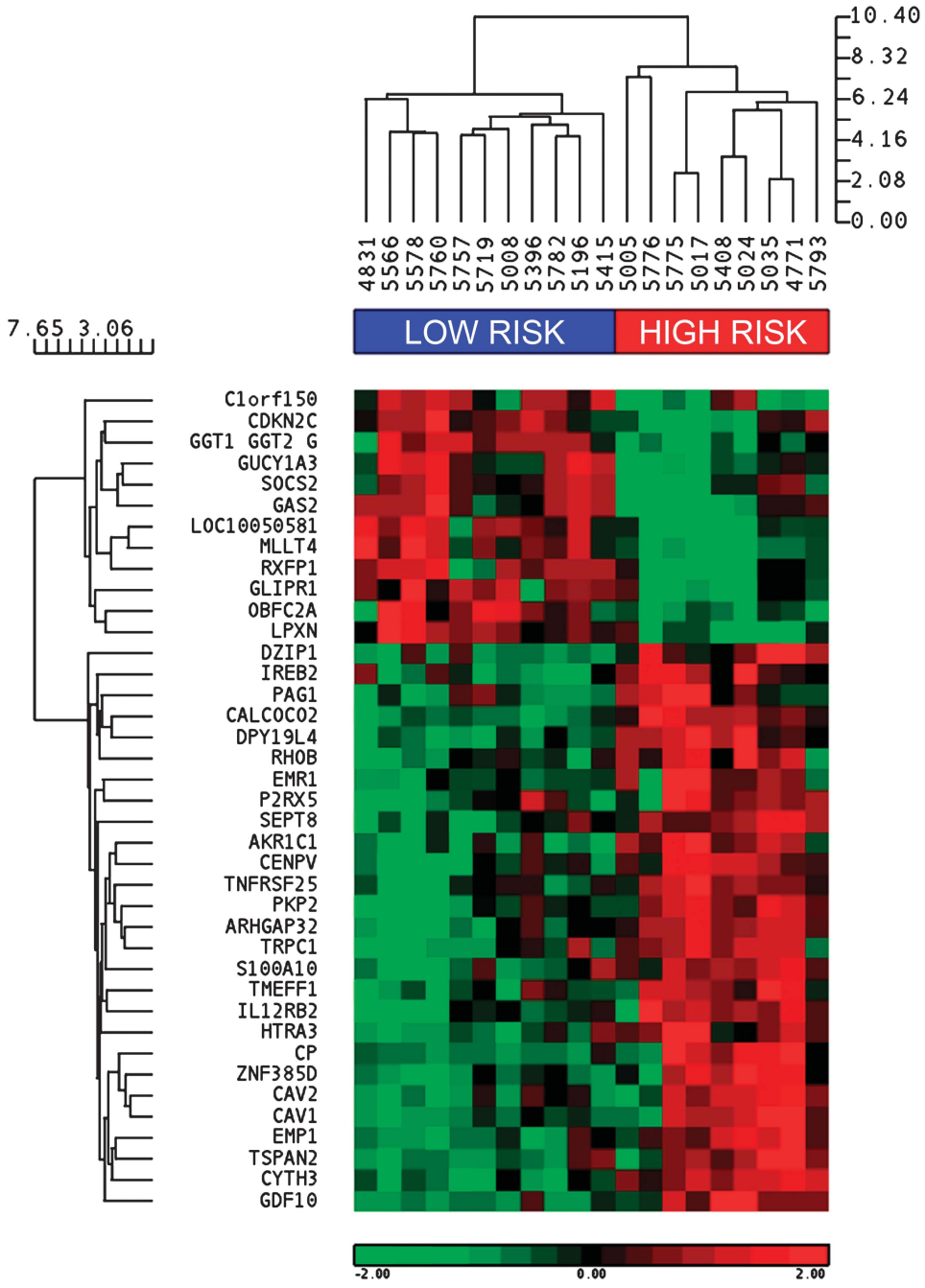


Figure 5. Gene expression profile was analyzed in CD34$^+$ cells purified from mutationally high-risk ($n=9$) and low-risk ($n=11$) patients randomly selected in the European cohort. The heat map was computed on the gene list provided in Supplementary Table 4 using the clustering algorithm included in the Partek GS package (Partek Incorporated, St Louis, MO, USA) by means of euclidean distance and average linkage. Gene coloring is based on normalized signals, as shown at the bottom of the figure; green indicates reduced expression, red increased expression. Gene symbol is indicated on the left. Low-risk and high-risk groups clustered separately in the dendrogram shown at the top of the heat map.

were considered separately from higher-risk categories (intermediate-2 plus high-risk patients) (Figures 3a and b).

Mayo Clinic cohort. Univariate analysis identified *ASXL1* (HR: 2.0, 95% CI: 1.5–2.7; $P<0.0001$), *SRSF2* (HR: 1.6, 95% CI: 1.1–2.4; $P=0.01$), *EZH2* (HR: 1.9, 95% CI: 1.1–3.4; $P=0.03$) and *IDH1* (HR: 2.5, 95% CI: 1.1–5.6; $P=0.02$) mutations as being prognostically detrimental for survival. Prognostic significance was sustained for all but *IDH1* mutations in multivariable analysis that included all four mutations. However, when each one of the four mutations was individually evaluated in the context of DIPSS-plus, only *ASXL1* mutations remained significant (HR: 1.4, 95% CI: 1.0–1.9; $P=0.04$). The same held true when all four mutations and DIPSS-plus were included in the multivariable model as covariates (HR: 1.4, 95% CI: 1.0–2.0; $P=0.046$). Notably, the prognostic relevance of *ASXL1* mutations was mostly restricted to patients with either intermediate-2 risk ($P=0.03$) or intermediate-1 plus low-risk disease ($P=0.07$) unlike high-risk disease ($P=0.78$) (Figure 4a).

Both univariate ($P=0.0004$ and $P=0.0001$, respectively) and multivariable (including karyotype and thrombocytopenia as

covariates; $P = 0.0002$ and $P = 0.02$, respectively) analysis for leukemia-free survival identified *SRSF2* and *IDH1* mutations as being significant. Considering the prognostic relevance of *ASXL1* mutations for survival and *SRSF2* and *IDH1* mutations for leukemia-free survival, the Mayo cohort was divided into mutationally high (*ASXL1*, *SRSF2* or *IDH1* mutated) and mutationally low (none of the above) risk categories, which showed significant difference in both overall (multivariable HR accounting for DIPSS-plus: 1.4, 95% CI: 1.0–1.9; $P = 0.04$) and leukemia-free (multivariable HR accounting for karyotype and thrombocytopenia: 3.2, 95% CI: 1.6–6.4; $P = 0.001$) survival. Significant differences in survival between the mutationally high- and low-risk disease groups were again not apparent in DIPSS-plus high-risk disease ($P = 0.39$) but were restricted to low plus intermediate-1 risk disease categories ($P = 0.02$; Figure 4b).

Association of 'mutationally high risk' status with a specific molecular signature

To explore whether these specific molecular assets associated with unique gene expression profiles, we compared $CD34^+$ cells from 9 mutationally high and 11 mutationally low-risk patients, randomly selected in the European cohort. As shown in the heat map in Figure 5, the two mutational groups displayed a distinct molecular signature, that was characterized by 39 differentially expressed genes, 27 overexpressed and 12 downregulated (Supplementary Table 4).

DISCUSSION

In this international collaborative project, we studied two independent cohorts of patients with PMF in order to clarify the prognostic relevance of newly described mutations, including *TET2*,[19] *CBL*,[20] *IDH1* or *IDH2*,[21] *ASXL1*,[22] *EZH2*,[23] *DNMT3A*[24] and *SRSF2*.[25] We used the first cohort of 483 European patients studied within 1 year of diagnosis, to select out which one of these mutations predicted worse outcome. Overall and leukemia-free survivals were inter-independently predicted by *ASXL1*, *SRSF2* or *EZH2* mutations and *ASXL1*, *SRSF2* and *IDH1* or *2* mutations, respectively. The observations in terms of overall survival were validated in an independent cohort of 396 Mayo Clinic patients studied at the time of their referral, whereas only *SRSF2* and *IDH1* mutations were associated with leukemic transformation in the Mayo cohort. When both cohorts were subjected to multivariable analysis that included recently developed prognostic models for survival in PMF (IPSS[3] in the European cohort and DIPSS-plus[10] in the Mayo cohort), only *ASXL1* mutations retained their significance. IPSS- or DIPSS-plus-independent prognostic value was also demonstrated by classifying patients into mutationally 'high' or 'low' risk groups defined as the respective presence or absence of at least one of the aforementioned mutations that were prognostically relevant for either overall or leukemia-free survival.

We believe that there is a major practical relevance of the observations from the current study in terms of disease prognostication and therapeutic decision making. The currently used scores for survival prognostication in PMF include IPSS,[3] DIPSS[9] and DIPSS-plus; the latter incorporates karyotype,[14,15] consistent with the major prognostic contribution of karyotype in other myeloid malignancies including AML[26] and MDS.[27] Conceivably, inv,[3] i(17q) or monosomal karyotype were identified as predictors of a $>80\%$ 2-year mortality in PMF.[28] In line with these observations, the current study identifies additional submicroscopic genetic changes, such as *ASXL1* mutations, which cluster with normal karyotype and yet portend a poor prognosis. Accordingly, whereas the outcome of patients with IPSS/DIPSS-plus high-risk disease may not be affected by the presence of *ASXL1* mutations, the apparently lower-risk patients might not fare as well as expected, an observation that might affect therapeutic decisions. Similarly, the presence of *SRSF2* or *IDH1* mutations appears to predict leukemia independent of currently known risk factors including thrombocytopenia and unfavorable karyotype.

The potential prognostic value of *IDH*,[29] *EZH2*[30] and *SRSF2*[31] mutations in PMF were previously suggested by single center studies. We reasoned that an integrated and comprehensive mutational analysis of the most commonly occurring mutations, performed in large well-characterized cohort of subjects with PMF, could allow to identify mutationally defined subgroups of patients resulting in improved prognosis assessment and providing a background for the interpretation of results obtained with conventional therapy, including allogeneic stem cell transplantation and particularly novel drugs. As was the case in the current study, *SRSF2*, *EZH2* and *IDH1* mutations have previously been associated with poor outcome in MDS[32–35] and *IDH1* in distinct subtypes of AML.[36–39] The detrimental effect of *ASXL1* mutations has also been shown in MDS,[34,35,40] AML[41–43] and CMML.[44] Therefore, *ASXL1* mutations represent a relatively frequent and independent prognostic biomarker in myeloid malignancies. Additional value is also suggested for *EZH2*, *IDH1* and *SRSF2* mutations. These observations suggest an untapped resource of genetic alterations for disease prognostication and warrant the inclusion of mutation screening for *ASXL1*, *EZH2*, *SRSF2* and *IDH1* as laboratory correlative studies in future clinical trials and prospective observational studies. Other potential prognostic biomarkers in PMF include nullizygosity for *JAK2* 46/1 haplotype,[45] low *JAK2*V617F allele burden,[46,47] increased serum IL-8 and IL-2R levels and excess serum-free light chain levels.[41,48]

The current study identifies mutations and mutational combinations that have clinical and prognostic correlates in PMF and could eventually prove useful for advancing the understanding of disease mechanisms, as suggested by the association of a specific gene expression signature in the $CD34^+$ cells of an exploratory cohort of subjects with their mutational asset. These results are also consistent with previous suggestions that mutations affecting epigenetic regulation might be prognostically more relevant than those involved with JAK-STAT signaling. Regardless, mutational profiling in PMF might clarify disease heterogeneity, refine current prognostic models, inform therapeutic decisions and provide clinical trials with a tool for better patient stratification and monitoring of the effects of novel drugs.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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