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Impact of High-Molecular-Risk Mutations on Transplantation Outcomes in Patients with Myelofibrosis

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Mutational profiling has demonstrated utility in predicting the likelihood of disease progression in patients with myelofibrosis (MF). However, there is limited data regarding the prognostic utility of genetic profiling in MF patients undergoing allogeneic hematopoietic stem cell transplantation (allo-HCT). We performed high-throughput sequencing of 585 genes on pre-transplant samples from 101 patients with MF who underwent allo-HCT and evaluated the association of mutations and clinical variables with transplantation outcomes. Overall survival (OS) at 5 years post-transplantation was 52%, and relapse-free survival (RFS) was 51.1 % for this cohort. Nonrelapse mortality (NRM) accounted for most deaths. Patient's age, donor's age, donor type, and Dynamic International Prognostic Scoring System score at diagnosis did not predict for outcomes. Mutations known to be associated with increased risk of disease progression, such as *ASXL1, SRSF2, IDH1/2, EZH2*, and *TP53*, did not impact OS or RFS. The presence of *U2AF1* (P = .007) or *DNMT3A* (P = .034) mutations was associated with worse OS. A Mutation-Enhanced International Prognostic Scoring System 70 score was available for 80 patients (79%), and there were no differences in outcomes between patients with high risk scores and those with intermediate and low risk scores. Collectively, these data identify mutational predictors of outcome in MF patients undergoing allo-HCT. These genetic biomarkers in conjunction with clinical variables may have important utility in guiding transplantation decision making.

INTRODUCTION

The introduction of ruxolitinib, a selective inhibitor of Janus kinase (JAK) 1 and 2, has significantly improved the outcomes of patients with myelofibrosis (MF) by reducing spleen size and constitutional symptom burden, along with improving overall survival (OS) [1,2]. However, current JAK inhibitors have limited anticlonal activity, and partial or complete remissions are not usually observed. Thus, allogeneic hematopoietic stem cell transplantation (allo-HCT) remains the sole potential curative treatment for patients with MF. Historical data indicate highly variable outcomes for patients with MF undergoing allo-HCT, and a variety of clinical factors have been identified as potentially impacting transplantation outcomes. Rondelli et al [3] reported that in patients who underwent allo-HCT from a related or unrelated donors after a reduced intensity conditioning (RIC) regimen, receipt of transplant from an unrelated donor, regardless of HLA match status, was associated with worse survival. In a different prospective study, Kroger et al [4] reported that older age (>55 years) and receipt of transplant from an HLA-mismatched donor were associated with worse survival. The differing clinical variables contributing to transplantation outcomes identified in previous studies highlights the need to develop

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robust predictive tools to better prognosticate outcomes in patients undergoing allo-HCT and to determine a given patient's appropriateness for allo-HCT.

The importance of genomic alterations in understanding the pathogenesis of myeloproliferative neoplasms (MPNs) has increased significantly in the past decade. Although activation of the JAK-STAT signaling pathway remains the hallmark of MPN pathogenesis, it has become clear that the presence of additional genomic events, such as mutations in *TET2* [5], *EZH2* [6], and *TP53* [7], alters the biology of disease in preclinical models. Furthermore, retrospective studies have demonstrated that the presence of select mutations may have important prognostic value in patients with MF. For example, the presence of *ASXL1, EZH2, SRSF2*, and *IDH1/2* mutations [8], as well as a lack of canonical *JAK2, MPL*, or *CALR* mutations (so-called "triple-negative" [TN] status) [9], are associated with an increased risk of leukemic transformation and poor survival, and the presence of any of these mutations is considered high-molecular-risk (HMR) disease. Moreover, the presence of certain genotypes appears to predict a less-durable response to JAK inhibitor therapy in patients with MF [10–12]. Thus, molecular genetic profiling offers important prognostic information in patients with MF. Indeed, integration of clinical variables and prognostic mutational data has recently resulted in the development of a novel prognostic tool, the Mutation-Enhanced International Prognostic Score System for transplantation-age patients with primary myelofibrosis (MIPSS-70) [13].

To date, limited data are available regarding the ability of genomic alterations to prognosticate outcomes in patients with MF undergoing allo-HCT [14]. To determine whether genomic alterations impact the outcome of patients with MF undergoing allo-HCT, as well as to determine how genomic alterations interact with other prognostically important disease- and transplantation-related factors [3,15–17], we performed comprehensive mutational profiling using a 585-gene panel in a multicenter cohort of patients with MF who underwent allo-HCT.

METHODS

Patients

This multicenter retrospective analysis comprised 101 patients diagnosed with primary MF or MF arising from another MPNs (ie, ET and PV) and undergoing HCT between 2007 and 2015. The study cohort included patients treated on the Myeloproliferative Disorders Research Consortium (MPD-RC) 101 prospective study (n = 52) (ClinicalTrials.gov; NCT00572897). A total of 49 patients with available pretransplantation molecular samples were collected from participating institutions, including 19 patients treated at Memorial Sloan Kettering Cancer Center in New York, 18 patients treated at Princess Margaret Cancer Centre in Toronto, and 12 patients at Moffitt Cancer Center in Tampa. Peripheral blood- or bone marrow aspirate-derived DNA before transplantation, as well as at the time of relapse in selected subjects, was available for sequencing from all patients. All samples were sequenced at one time point retrospectively, and thus molecular data were not available for the treating physicians when transplantation decisions were made. Approval for the study was obtained from the Institutional Review Boards of Memorial Sloan Kettering Cancer Center and of all participating institutions, in accordance with the Declaration of Helsinki.

Sample Processing, Sequencing, and Mutation Analysis

We performed high-throughput sequencing with a targeted deep sequencing assay of 585 genes (HemePACT) as described previously [18]. In brief, tumor tissue (peripheral blood or bone marrow aspirate) was sequenced at an average coverage of 829× (with a standard deviation of 130). The reads were aligned to the human genome (UCSC build hg19) using the Burrows-Wheeler aligner with maximal exact matches [19]. We used the Cancer Genome Project pipeline [20] to compare the tumor samples with a standard cancer-free germline following the pipeline recommendations. Snpeff [21] was used to annotate variants with functional consequence on genes. We filtered out common population germline variants using the ExAC dataset [22]. We only considered variants that were either present in at least 2 samples or classified as oncogenic or likely oncogenic following criteria published by Papaemmanuil et al [23]. The lower limit of detection of the assay used in this analysis is a variant allele fraction (VAF) of .5%.

Statistical Analysis

The overall survival (OS) and relapse-free survival (RFS), in which relapse and death were defined as event of interest, for the whole cohort were estimated using Kaplan-Meier method. To investigate the association between clinical characteristics (10 demographic and clinical variables prespecified) and gene mutations (22 individual genes with gene mutation frequency >1%, and 4 groups of gene variables including TN MPN, presence of HMR mutations, MPN driver mutation groups, and presence of 3 or more somatic mutations) and OS as well as RFS outcomes, univariate Cox regression was used to estimate the hazard ratio for each potential predictor. All the potential predictors (total of 36 variables) were put in the multiple Cox regression model and forward selection using .1 as the significant level was used to choose the final variables in the multivariable model. Nonrelapse mortality (NRM) and cumulative incidence of relapse were estimated using Fine and Gray's method [24] in the presence of competing risk (ie, relapse as competing risk for NRM and death without relapse as competing risk for relapse). A proportional hazards model for the subdistribution of NRM was used to estimate the hazard ratio (HR) for each potential predictor for NRM. SAS 9.3 (SAS Institute, Cary, NC) was used to analyze the data.

RESULTS

Genomic Analysis of Pretransplantation MF Cohort

High-throughput sequencing using a panel of 585 cancer-related genes was performed on peripheral blood and bone marrow samples obtained before allo-HCT as described above (Figure 1A and **Supplementary Table 1**). The majority of patients had an activating *JAK2* mutation (56.4%). Mutations in chromatin modifiers (*ASXL1*, 18% and *EZH2*, 4%), as well as splicing factors (*SRSF2*, 12%; *U2AF1*, 10%; *SF3B1*, 4%), were the most frequently observed class of non-JAK-STAT mutations in this cohort. Less-frequent mutations were identified in genes involved in DNA methylation regulation, such as *IDH2* and *TET2* (8% each) and *DNMT3A* (5%). Notably, we identified recurrent mutations in *KMT2C* in 11% of patients.

As mentioned above, the presence of mutations in *ASXL1, SRSF2, IDH1/2*, and *EZH2* have been previously associated with an increased risk of leukemic transformation, and *TP53* mutations are enriched in post-MPN AML [8]. Collectively, these HMR mutations occurred in 36.6% of patients in this cohort. Lack of an identifiable JAK-STAT driver mutation (ie, TN status) was identified in 22 patients (21.8%). Fifty-one patients (50.5%) had either HMR risk status, TN status, or both. Thus, this cohort of patients was highly enriched for high-risk genomic alterations. Furthermore, previous data indicate that increasing numbers of mutations per patient are associated with an increased risk of leukemic transformation and impaired survival [25]. Sixty-two patients (61.4%) in this cohort had more than 1 mutation, inclusive of the driver mutation (Figure 1B).

Cytogenetic data were available for 86 patients (85%) in the cohort. Unfavorable cytogenetics were found in 24.7% of the patients, and 31.3% of the evaluable patients did not have any cytogenetic abnormalities (Figure 1C). The most common cytogenetic abnormality was del20q, identified in 18.6% of patients. A complex karyotype was identified in 3 patients.

Analysis of co-occurrence of mutational events and karyotype did not reveal a statistically significant association between any individual mutations and cytogenetic abnormalities (data not shown).

Impact of Clinical, Genetic, and Treatment Factors on Transplantation Outcomes

The median age of the cohort was 59 years (range, 30 to 73.4 years). Fifty-six patients (55.5%) had a Dynamic International Prognostic Scoring System (DIPSS) risk score of intermediate-2 or higher. MIPSS-70 score was available for 80 patients (79%), including 3 patients with low-risk, 29 with intermediate-risk, and 48 with high-risk scores. Sixty-nine patients had splenomegaly present at the time of transplantation (68.3%), and 11 patients

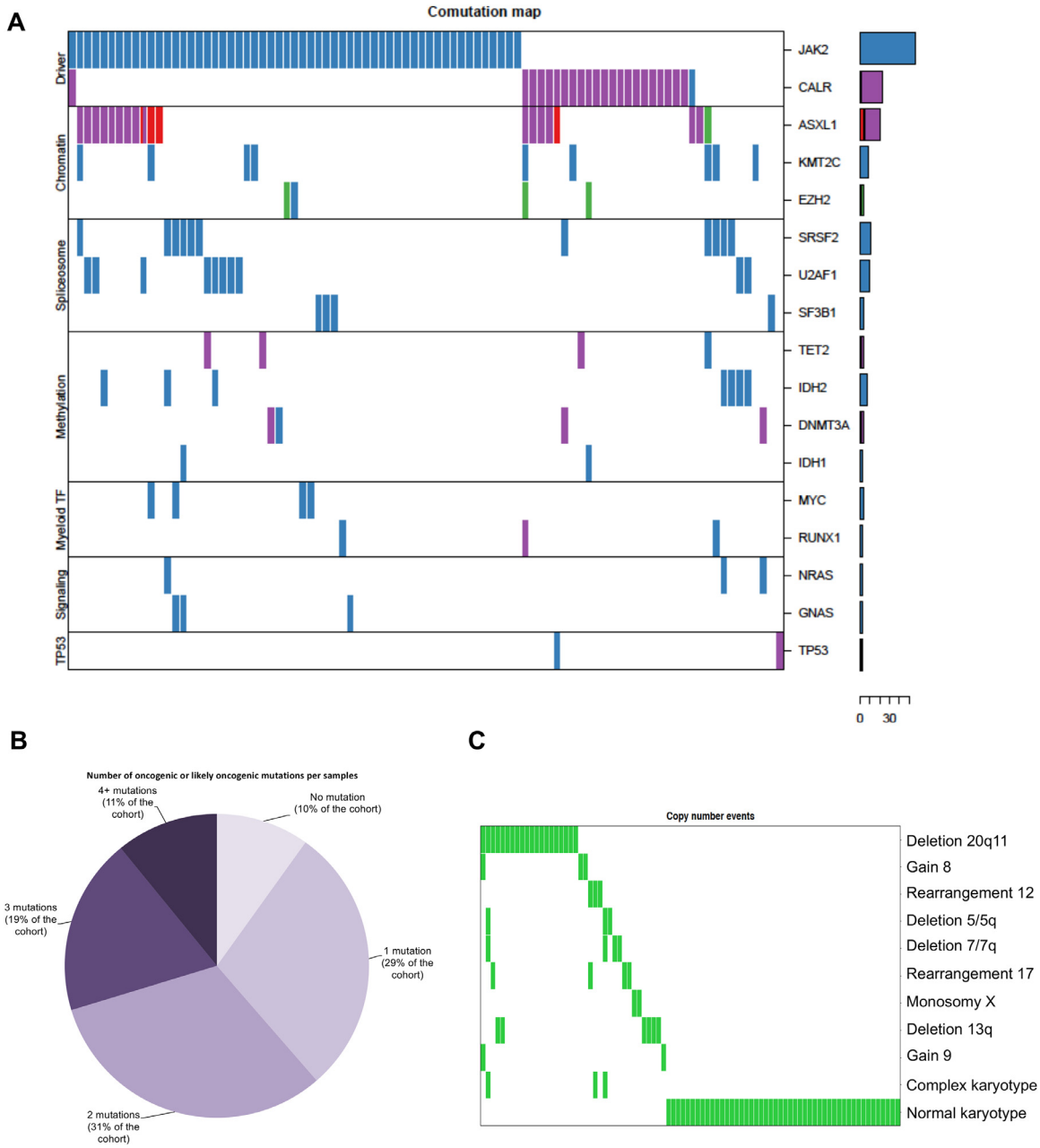

Figure 1. Summary of mutations and cytogenetic abnormalities detected in 101 patients with MF. (A) Spectrum and frequency of mutations. Mutations are grouped according to mechanism. (B) Number of mutations per sample. (C) Summary of cytogenetic data, which were available for 86 patients.

(10.9%) had undergone previous splenectomy. A matched donor was used in 98 of the 101 transplantations (46 related and 52 unrelated). The majority of patients received an RIC regimen (Table 1).

The median duration of follow-up for the entire cohort was 972 days (2.6 years; 95% confidence interval [CI], 770 to 1124 days). The OS for the cohort was 57.5% (95% CI, 48.0% to 68.8%) at 3 years and 52.0% (95% CI, 41.5% to 65.3%) at 5 years post-transplantation (Figure 2A). The RFS was 51.1% (95% CI, 41.6% to 62.8%) at 3 and 5 years post-transplantation (Figure 2B). Notably, nonrelapse mortality (NRM) accounted for the majority of deaths in the cohort; the cumulative incidence of NRM was 25.9% (95% CI, 18.6% to 36.2%) at 1 year post-transplantation and 39.0% (95% CI, 30.1% to 50.7%) at 3 and 5 years post-transplantation, and the cumulative incidence of relapse was 7.0% (95% CI, 3.4% to 14.3%) and 9.7 (95% CI, 5.1% to 18.3%), respectively (Figure 2C and D). The most common cause of death in this cohort was graft-versus-host disease (GVHD; 28.5%).

We examined the impact of patient-related characteristics, such as age, sex, and disease risk (by DIPSS), as well as transplantation-related characteristics, such as conditioning, donor type, and donor age, on overall outcomes for patients in this cohort. Patients who received an RIC regimen had worse OS compared with those who received a myeloablative conditioning (MAC) regimen (HR, 5.94; 95% CI, 1.43 to 24.62; $P = .005$) (Table 2 and Figure 3A) in a univariate analysis. Comparison of the MAC and RIC groups showed no statistically significant differences between the groups in terms of patient age, sex, cytogenetic risk group, DIPSS, time from diagnosis to transplantation, number of mutations, and presence of high-risk mutations, but the MAC

Table 1
Disease and Transplantation Characteristics

	(N=101)
Age at transplant	
Median, Range	59 (30.0-73.4)
< 50	13 (12.9%)
50 - 65	75 (74.3%)
> 65	13 (12.9%)
Gender: Male	60 (59.4%)
Diagnosis	
PMF	62 (61.4%)
Post ET MF	20 (19.8%)
Post PV MF	18 (17.8%)
MPN-U	1 (1.0%)
DIPSS	
Low Risk	9 (8.9%)
Int-1	36 (35.6%)
Int-2	41 (40.6%)
High Risk	15 (14.9%)
MIPSS-70	
Missing	21
High Risk	48 (60.0%)
Intermediate Risk	29 (36.3%)
Low Risk	3 (3.8%)
Cytogenetics	
Missing	15
Favorable	61 (70.9%)
Unfavorable	25 (29.1%)
3 or more somatic mutations	
Yes	30 (29.7%)
HMR: Presence of one of the mutations ASXL1/SRSF2/IDH1/2/EZH2/TP53	
Yes	37 (36.6%)
MPN Triple Negative (no for JAK2,MPL and CALR)	
Yes	22 (21.8%)
Spleen status	
Splenectomy	11 (10.9%)
Splenomegaly	69 (68.3%)
No splenomegaly	21 (20.8%)
Time from Diagnosis to Transplant (years)	
Median, range	1.9 (0.1-28.4)
Donor	
MRD	46 (45.5%)
MUD	52 (51.5%)
Mismatch	3 (3.0%)
Donor age	
Missing	17
Median, Range	45.5 (18.0-73.0)
Conditioning Regimen	
MAC	18 (17.8%)
RIC	83 (82.2%)

group had a higher proportion of mismatched donors (16.7% versus 0%; $P = .008$) (Supplementary Table 2). The majority of patients conditioned with an MAC regimen received a T cell-depleted (TCD) transplant (ex vivo $CD34^+$ selected allograft [26]) (13 of 18; 72.2%). In this analysis, patient age and graft source (related versus unrelated) had no impact on outcomes, in contrast to previous reports [3,4] (Table 2).

We next sought to determine the impact of molecular and cytogenetic parameters on survival by univariate analysis. The total number of mutations per patient was not associated with increased mortality risk (HR for mortality with ≥ 3 mutations versus <3 mutations was 1.22; 95% CI, .64 to 2.31; $P = .546$), indicating that allo-HCT may be able to overcome the poor prognostic impact of multiple mutations in patients with MPNs. Furthermore, the presence of HMR mutations did not impact the survival of patients in this cohort (HR for mortality with HMR mutation versus no HMR mutation, 1.42; 95% CI, .77 to 2.61; $P = .2603$) (Figure 3B). An analysis of the impact of individual mutations revealed that the presence of *U2AF1* or *DNMT3A* mutation was associated with worse OS (*U2AF1*: HR

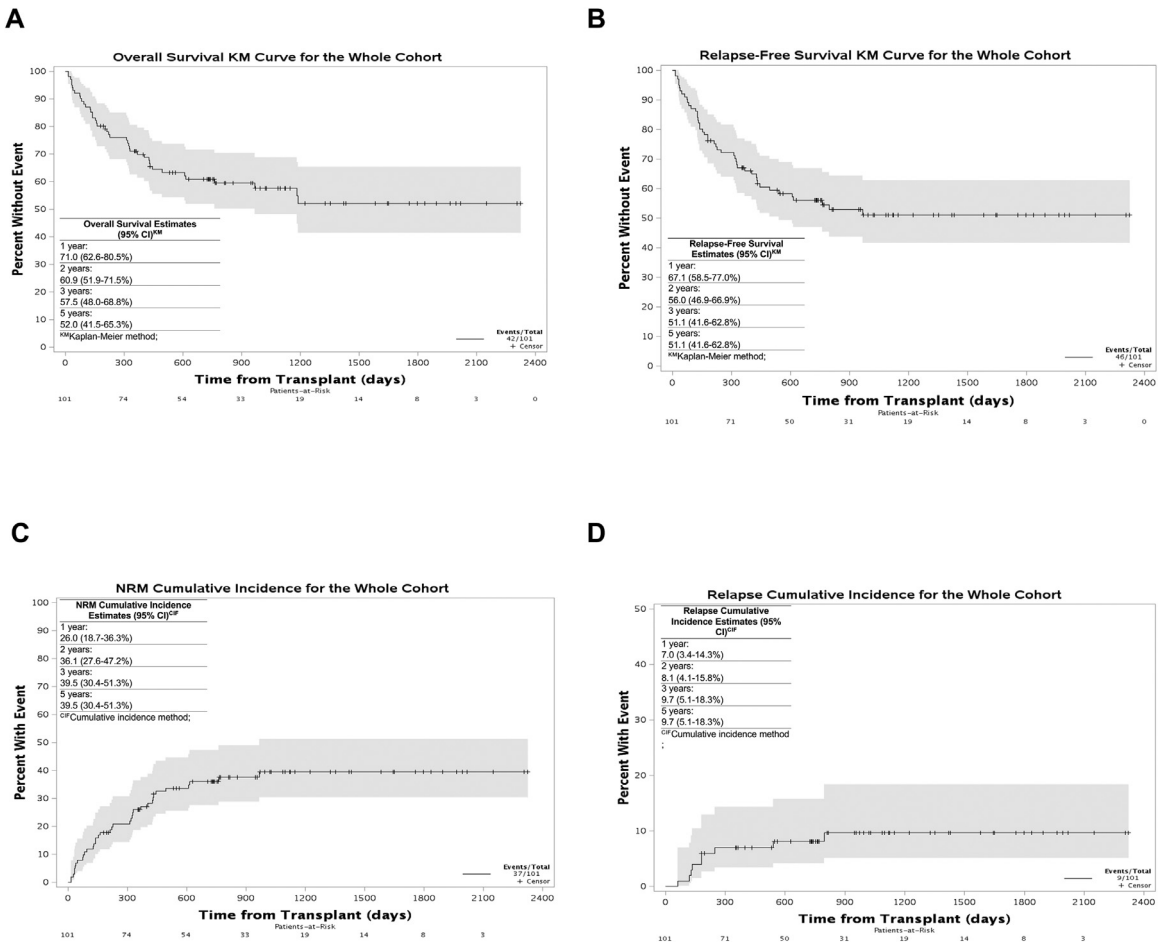

Figure 2. Kaplan-Meier curves for the whole cohort. (A) OS. (B) RFS. (C) NRM. (D) Cumulative incidence of relapse.

for death, 2.76; 95% CI, 1.28 to 5.99; P = .007; *DNM3TA*: HR for death, 2.91; 95% CI, 1.03 to 8.24; *P* = .034) (Figure 3C and D). Notably, 3 of the 4 deaths due to graft failure occurred in patients with a *U2AF1* mutation. In addition, the presence of a *U2AF1*, *DNMT3A*, or *IDH2* mutation was associated with an increased risk of NRM (**Supplementary Table 3**). Recently reported data indicate that the VAF of mutant genes, such as *TP53* [27], in myelodysplastic syndrome may impact the clinical outcomes of patients. Assessment of the impact of VAF of the most common mutations in this cohort (*JAK2, CALR*, and *ASXL1*), stratified by median VAF, did not demonstrate an impact on survival or relapse.

Analysis of the impact of cytogenetics categorization (as defined in the DIPSS-Plus scoring system) [28] demonstrated worse OS in patients with unfavorable cytogenetic abnormalities, with a trend toward significance (HR, 2.01; 95% CI. 1.01 to 4.00; *P* = .05) (Figure 3E).

An MIPSS-70 score was available for 79% of the patients in this cohort. Patients with intermediate-risk and high-risk scores composed the majority of the cohort (96%). Notably, there were no differences in transplantation outcomes between patients with high-risk scores and those with intermediate-risk scores (Table 2).

In multivariate analysis, both receipt of an RIC regimen (HR, 5.38; 95% CI, 1.29 to 22.39; *P* = .02) and the presence of a *U2AF1* mutation (HR, 2.83; 95% CI, 1.29 to 6.19; *P* = .009) remained negatively associated with OS. *DNMT3A* was also associated with worse survival, although this association did not reach statistical significance (HR, 2.78; 95% CI, 0.97 to 8.0; *P* = .057) (Figure 4).

With regard to RFS, analysis of clinical factors, molecular mutations, and cytogenetics demonstrated similar patterns to those seen with OS. Univariate analysis demonstrated that receipt of an RIC was associated with worse RFS compared with receipt of a MAC regimen (HR, 2.96; 95% CI, 1.06 to 8.26; *P* = .03), and that the presence of a *U2AF1* mutation (HR, 2.37; 95% CI, 1.10 to 5.08; *P* = .026) or a *DNMT3A* mutation (HR, 4.02; 95% CI, 1.56 to 10.35; *P* = .0018) was associated with worse RFS (**Supplementary Table 4 and Supplementary Figure 1**). In multivariate analysis, only *U2AF1* and *DNMT3A* mutations retained a significant association with reduced RFS, although there was a strong trend toward association of a RIC regimen as well (*P* = .0598) (**Supplementary Figure 2**).

Genomic Analysis of Post-Transplantation Relapse Samples

Among patients who relapsed following allo-HCT, 6 patients had available pretransplantation and at the time of relapse on which analysis of paired samples was carried out. This analysis demonstrated that the in many cases, relapsed sample contained the same clonal architecture as the pretransplantation samples (Figure 5A). In only 1 patient (patient 4) was a new mutation detected at the time of relapse. Analysis of chimerism over time following transplantation demonstrates that in many cases, loss of donor engraftment is detected before detection of the *JAK2*V617F allele (Figure 5B and **Supplementary Table 5**).

Table 2
Univariate Analysis of Clinical Characteristics and Mutation Analysis for OS

Effect	Level	HR (95% CI)	P value
Age at transplant	50 - 65 vs. < 50	1.10 (0.43,2.84)	0.9635
	> 65 vs. < 50	1.01 (0.31,3.31)	
Gender	F vs. M	0.95 (0.51,1.76)	0.8613
Cytogenetic risk	unfavorable vs. favorable	2.01 (1.01,4.00)	0.0547
	NA vs. favorable	2.19 (0.96,4.98)	
DIPSS	High Risk vs. Low Risk	1.33 (0.40,4.42)	0.4426
	Int-1 vs. Low Risk	1.24 (0.42,3.68)	
	Int-2 vs. Low Risk	0.73 (0.24,2.24)	
Spleen status	Splenectomy vs. No splenomegaly	1.95 (0.67,5.63)	0.4527
	Splenomegaly vs. No splenomegaly	1.43 (0.65,3.14)	
Conditioning intensity	RIC vs. MAC	5.94 (1.43,24.62)	0.0052
Time from diagnosis to transplant	>2 years vs. <= 2 years	1.07 (0.58,1.95)	0.8363
Primary vs seconday MF	Other dx vs. PMF	0.75 (0.40,1.43)	0.3816
Donor	Unrelated vs. Related	1.59 (0.85,2.96)	0.1436
Donor age	>=50 vs. <50	0.91 (0.46,1.80)	0.2808
	NA vs. <50	0.46 (0.18,1.22)	
Mutations	At least one positive vs. triple negative	1.22 (0.56,2.64)	0.6145
HMR presence	Yes vs. No	1.36 (0.73,2.56)	0.3334
3 or more somatic mutations	Yes vs. No	1.22 (0.64,2.31)	0.5467
JAK2	Yes vs. No	1.34 (0.71,2.53)	0.3572
CALR	Yes vs. No	0.72 (0.32,1.63)	0.4328
ASXL1	Yes vs. No	1.39 (0.67,2.92)	0.3755
SRSF2	Yes vs. No	0.95 (0.37,2.42)	0.9174
KMT2C	Yes vs. No	0.78 (0.28,2.19)	0.6342
U2AF1	Yes vs. No	2.76 (1.28,5.99)	0.0071
TET2	Yes vs. No	1.60 (0.63,4.08)	0.317
IDH2	Yes vs. No	2.23 (0.94,5.29)	0.0626
DNMT3A_cat	Yes vs. No	2.91 (1.03,8.24)	0.0345
MIPSS-70	High Risk vs. Intermediate/low risk	1.25 (0.62,2.52)	0.5372

DISCUSSION

Molecular genetic and cytogenetic analyses have been merged with analysis of clinical parameters to develop tools for prognostication in MF. Furthermore, molecular profiling has identified ruxolitinib-treated patients with decreased time to treatment failure [10], thus allowing for prediction of patients at risk of poor response to ruxolitinib. In contrast, few predictive models exist for patients with MF being considered for allo-HCT, thus complicating treatment decisions for physicians and patients, particularly given the risks of allo-HCT. Therefore, we sought to extend the impact of mutational profiling as a prognostic tool to patients undergoing allo-HCT.

In multivariate analysis, mutations previously associated with worse outcomes in patients with MF, such as *ASXL1, EZH2, SRSF2, IDH1/2,* and *TP53* mutations, were not found to affect OS or RFS in patients with MF undergoing allo-HCT. Furthermore, the number of mutations per patient did not impact OS or RFS. These findings suggest that allo-HCT can overcome the poor prognosis associated with these mutations. It may further imply that patients with HMR or those who are likely to have short duration of benefit from ruxolitinib should be referred for earlier allo-HCT evaluation. We identified *U2AF1* mutations as a risk factor for decreased OS, and both *U2AF1* and *DNMT3A* mutations were associated with impaired RFS. Mutations in *U2AF1* have been reported in approximately 10% to 15% of patients with MF and have been shown to strongly correlate with the degree of anemia [29,30] and also with worse OS compared with patients with unmutated *U2AF1*. Interestingly, in our cohort, 4 patients died secondary to graft failure, 3 of whom had a *U2AF1* mutation. We were unable to identify other factors related to disease, donor, or transplant that put these patients at greater risk for graft failure compared with the rest of the cohort. All 4 patients received an RIC regimen and received a transplant from a matched unrelated donor. Two patients had DIPSS intermediate-1 disease, and 2 patients had intermediate-2 disease. This raises the possibility of cell nonautonomous effects of cells bearing mutant *U2AF1* on the bone marrow microenvironment. *DNMT3A* mutations appear to mediate

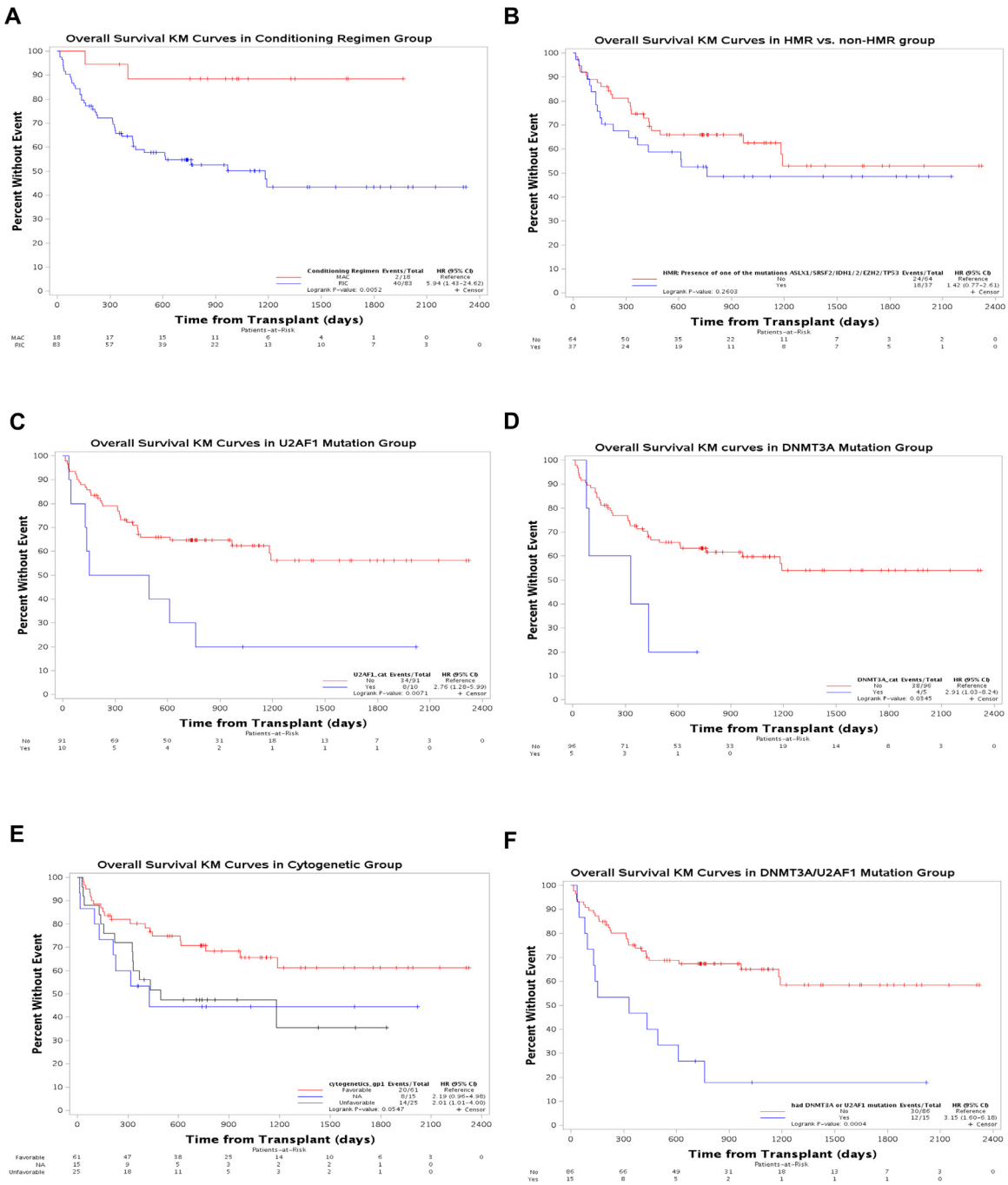

Figure 3. Kaplan-Meier curves for OS by conditioning intensity, mutations, and cytogenetic abnormalities. (A) OS with the use of MAC or RIC. (B) OS in the presence or absence of HMR mutations. (C) OS in the presence or absence of *U2AF1* mutations. (D) OS in the presence or absence of *DNMT3A* mutations. (E) OS in the presence of favorable and unfavorable cytogenetic abnormalities. (F) OS under the combined effect of conditioning intensity and presence or absence of *U2AF1* and *DNMT3A* mutations.

anthracycline-based chemotherapy resistance in AML and *DNMT3A* R882 in particular is predictive of minimal residual disease in AML [31]. Thus, it is possible that the presence of *DNMT3A* mutations renders MPN hematopoietic stem cells relatively resistant to the effects of conditioning. Thus, the biological impact of *U2AF1* and *DNMT3a* mutations may affect the likelihood of transplantation success. Further genomic and biological studies are needed to validate these observations.

The mutational profile of our cohort was similar to previous reports of patients with MF reported in the literature. However, we did identify mutations in *KMT2C*. *KMT2C* mutations have been described in various solid tumors [32–35] and were recently reported by Durham et al [36] in classical and variant hairy cell leukemia. In addition, Chang et al [37] recently reported *KMT2C* mutations in a group of patients with TN MPN. The biological contribution of *KMT2C* mutations to MPN pathogenesis remains to be determined, however.

Most cases of mortality in this cohort were not related to relapse, and indeed the incidence of relapse was surprisingly low despite the fact that 55% of the patients had advanced disease (intermediate-2 and high-risk disease), and many patients had HMR mutations. In contrast, data from the MDS and AML

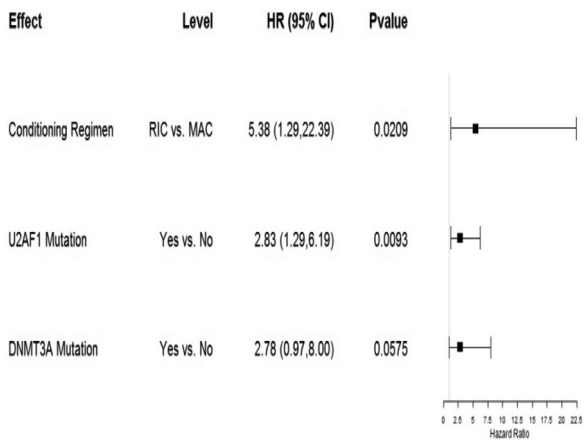

Figure 4. Multivariate analysis for OS as a forest plot.

literature indicates that certain mutations are predictive of very poor prognosis after allo-HCT, mostly due to disease relapse [38,39]. Moreover, our findings contrast with data recently published by Kroger et al [14] demonstrating an association between *ASXL1* mutations and greater risk of relapse. In our cohort, among 19 patients with an *ASXL1* mutation, 9 patients died without relapse at a median of 4 months post-transplantation. Differences between the cohorts, and the resulting differences in RFS and NRM, may account for differences in the observed impact of mutations on outcomes. Thus, larger cohorts of patients are needed to validate observations from these studies.

A recent analysis reported by Wolschke [40] et al examined the impact of minimal residual disease by molecular studies after allo-HCT in patients with MF. This study demonstrated that patients with persistent evidence of disease at the molecular level at day 100 or 180 after allo-HCT had a significantly higher relapse incidence compared with patients without molecular residual disease (62% versus 10%). In congruence with this observation, we detected the same mutational profile pretransplantation and post-transplantation in most patients who sustained relapse, without evidence of clonal evolution. In 2 patients, loss of clones containing *ASXL1* and *KMT2C* mutations were noted, suggesting some degree of selective pressure by allo-HCT on different subclones. These observations suggest that mutational analysis may play an important adjunctive role (together with chimerism analysis) in minimal residual disease monitoring. Further studies of depth of molecular response are required to define clinically meaningful molecular minimal residual disease.

The majority of patients in this retrospective analysis were conditioned with an RIC regimen, and an MAC regimen was used mostly in the context of TCD transplants (ex vivo positive selection of $CD34^+$ stem cells using the CliniMACS CD34 Reagent System [26]). The use of MAC was associated with better OS in this analysis, which could not be accounted for by patient baseline characteristics. Historically, patients with MF who underwent allo-HCT with a MAC regimen had a high incidence of NRM, and thus most patients with MF are offered an RIC. It is important to note that the MAC regimen used with TCD transplants was chemotherapy-based and did not include total body irradiation. This may explain the better outcomes compared with those historically reported with MAC regimens [16,41]. It is also possible that with a TCD transplant, the lack of need for calcineurin inhibitors for GVHD prophylaxis, as well as the lower incidence of GVHD in these patients, accounted for the better outcomes compared with what has been historically reported with MAC in patients with MF. These finding are important in the context of the MAC versus RIC study [42] in patients with MDS and AML, in which the MAC regimen was superior in patients with AML. We also recognize the limitations associated with interpreting our findings when using a small cohort of patients, and we believe that further prospective studies addressing the intensity of conditioning regimen in patients with MF are important.

Our data establish that genomic alterations have predictive value with regard to allo-HCT and are likely useful in guiding transplantation decision making in patients with MF. It also suggests that mutations associated with poor prognosis and progression to AML are not predictive of post-transplantation outcomes. Moreover, these observations raise new questions about how genomic alterations may impact transplantation

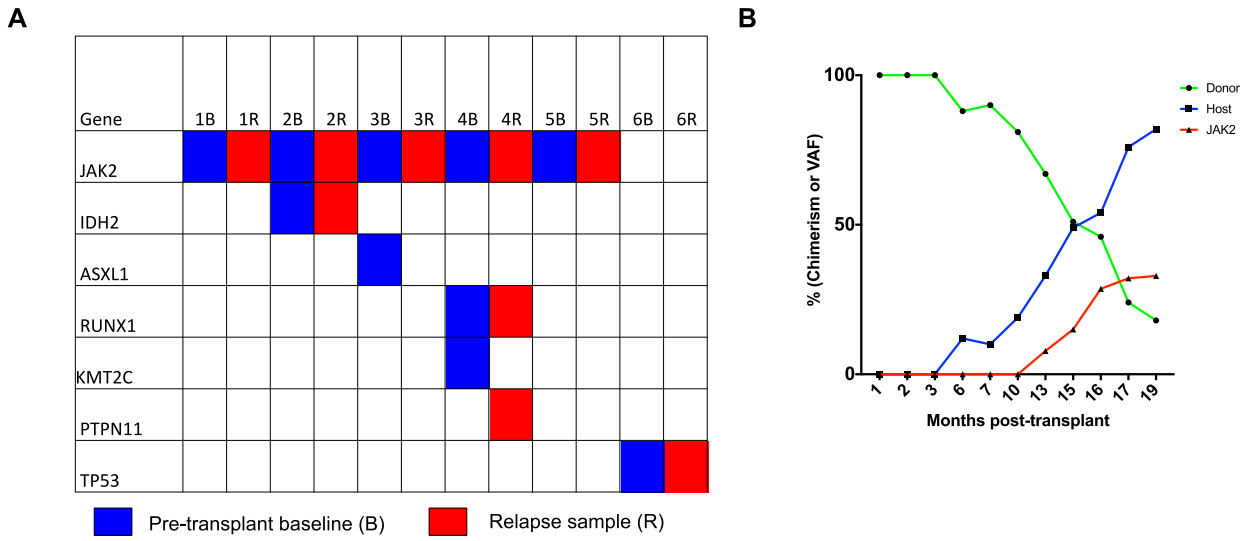

Figure 5. Mutational analysis of cases of disease relapse post-transplantation. (A) Sequencing analysis of 6 paired pretransplantation and post-transplantation relapse cases. (B) Trend over time of chimerism and recurrence of *JAK2*V617F mutation post-transplantation.

outcomes in patients with MF and whether interventions to eliminate the mutated clone, particularly in patients with mutated *U2AF1*, will impact transplantation outcomes. Notably, a clinical trial of inhibitors targeting splicing factors is currently underway (ClinicalTrials.gov; NCT02841540). Considering the rarity of MF and the relatively small numbers of allo-HCTs performed for this disease, we strongly believe that further analyses with larger cohorts are needed to confirm the findings of this analysis. Finally, prospective studies are needed to assess the optimal conditioning regimen in patients with MF.

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SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at doi:10.1016/j.bbmt.2019.01.002.

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