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The number of additional high molecular risk mutations predicts outcome after hematopoietic stem cell transplantation in primary and secondary myelofibrosis

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To the Editor,

Defining prognosis in myelofibrosis (MF) is of particular clinical relevance since survival may be highly heterogeneous, ranging from years to a few months. The biological landscape knowledge of MF has evolved in recent years, with the discovery of additional mutations in the myeloid-related genes beyond driver mutations. Mutations in *ASXL1, EZH2, SRSF2, IDH1/2* and *U2AF2* genes are defined as high-molecular risk mutations (HMR) [1, 2], and their presence has been associated with poorer survival. Consequently, the presence of HMR mutations has been incorporated into various prognostic scores and helps in defining prognosis, driving therapeutic strategies.

Available data specifically addressing the impact of molecular factors on transplant outcome are mostly limited to primary myelofibrosis and have not shown a prognostic impact of the presence of HMR mutations on post-transplant outcome [3–6]. Only *ASXL1* mutations, *JAK2V617F* mutation/triple negativity [7] or the presence of multi-hit mutations of *TP53* [8] were associated with a poorer survival.

This study aimed to analyze the effect of the "number" of additional detrimental mutations included in the HMR category on transplant outcome in patients with primary or secondary myelofibrosis undergoing allogeneic stem cell transplantation.

We retrospectively analyzed 50 consecutive patients aged 36–67 years with primary or secondary MF in chronic phase, who underwent allogeneic transplantation between September 2007 and September 2023. Seventy-five percent of the transplants were performed after 2015. All patients included in this study signed an informed consent form, which allowed their data to be transferred to the national competent authority, the EBMT (European Bone Marrow Transplantation) Registry, and to be used for scientific purposes. This study was conducted following the Declaration of Helsinki. Baseline patient, disease and transplant characteristics are summarized in Table 1. Next-generation sequencing (NGS) analysis was available for all patients (Supplementary Table S1 and Supplementary Fig. S1) and revealed at least an additional mutation in 44 patients; 28 patients (56%) had at least one high-molecular risk mutation (HMR), and ten patients (20%) had two or more HMR mutations. Patients' risk classification according to the current prognostic scores is reported in Supplementary Table S2. Details on the applied treatments and statistical analyses are given in the Supplementary Information.

With a median follow-up of 1.9 years (range 0.1–14.5 years), the 3-year overall survival (OS) and progression-free survival (PFS) were 58 and 50%. The 3-year cumulative incidence of relapse (CIR) was 27%, while the 3-year cumulative incidence of non-relapse mortality (NRM) was 24%. The 3-year OS, PFS, and NRM were 67, 56, and 17% for patients with none or only one HMR mutation and 20, 20, and 50% for patients with ≥2 HMR mutations, respectively (Fig. 1A–C).

By univariate analysis (Supplementary Tables S3, S4), very high-risk karyotype negatively impacted OS (HR 3.28, $p = 0.04$) and PFS (HR 4.76, $p = 0.002$). Triple negativity was associated with inferior PFS (HR 5.87, $p = 0.006$) and higher CIR (HR 14.4, $p < 0001$), while *JAK2V617* mutation was associated with a trend for a higher CIR (HR 4.26, $p = 0.18$). With regards to additional mutations, the presence of an HMR mutation per se did not impact outcome, while the presence of 2 or more HMR mutations was associated with inferior OS (HR 4.23, $p = 0.002$), PFS (HR 3.45, $p = 0.005$) and NRM (HR 4.13, $p = 0.018$). We next evaluated the individual effect of additional mutations, and we found a significant impact on outcome for *DNMT3A* (inferior OS and PFS), *EZH2* (inferior OS, PFS, and higher NRM) and *TP53* (higher CIR), while we could not observe any impact on outcome for *ASXL1*.

Concerning pre-transplant clinical characteristics, severe thrombocytopenia (PTL <50.000/mcL) was remarkably associated with inferior OS (HR 5.05, $p = 0.004$) and PFS (HR 3.94, $p = 0.01$), while severe anemia (Hb < 8 g/dl) was associated with worse NRM (HR 3.58, $p = 0.048$). A higher NRM was seen in patients with a longer interval between diagnosis and transplant (HR 1.13, $p = 0.059$).

Donor bone marrow chimerism <95% at 30 days after transplant was significantly associated with PFS (HR 2.98, $p = 0.03$) and with CIR (HR 8.07, $p = 0.0004$), without an impact on OS. Lastly, we could not find any effect on the outcome for age at transplant, splenomegaly >22 cm, splenectomy, intensity of conditioning, HCT-I index, splanchnic vein thrombosis, and donor type.

Multivariate analysis (Supplementary Table S5) confirmed that the presence of 2 or more high-molecular risk mutations negatively impacted OS (HR 34.5, $p = 0.002$), PFS (HR 46.4, $p = 0.0009$), and NRM (HR 144, $p = 0.028$). The presence of mutations in *DNMT3A* was associated with a reduced OS (HR 5.48, $p = 0.03$) and PFS (HR 5.17, $p = 0.02$), while triple negativity maintained the negative impact on CIR (HR 23.9, $p < 0.0001$). Pre-transplant severe thrombocytopenia retained a negative impact on OS (HR 11.36, $p = 0.005$), while severe anemia on NRM (HR 8.78, $p = 0.0007$). Finally, donor bone marrow chimerism<95% at day +30 was consistently associated with worse PFS (HR 5.91, $p = 0.016$) and CIR (HR 8.75, $p = 0.002$).

Previous studies explored the prognostic role of additional mutations on transplant outcome. Kroger et al. [3] analyzed the impact of *ASXL1, CALR, SRSF2, TET2, U2AF1, EZH2, MPL, IDH1/2*, and *CBL* mutations, observing that only *ASXL1* and *IDH2* mutations were independent risk factors for lower PFS. Additionally, Tamari et al. [4] found that the presence of *ASXL1, SRSF2, IDH1/2, EZH2*, and *TP53* did not impact OS or relapse-free survival, while the presence of *U2AF1* or *DNMT3A* mutations was associated with worse OS. In line with these findings, our results confirm that the presence of the HMR category itself does not affect transplant outcome.

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Table 1. Patient, disease and transplant characteristics.

Characteristics at transplant	All, $n = 50$ (%)
Median age, years (range)	58.5 (36–67)
Sex, *N*(%)	
Female	14 (28)
Males	36 (72)
Myelofibrosis, *N*(%)	
Primary	17 (34)
Secondary to PV/ET	33 (66)
ET	21
PV	12
Anemia (Hb <10 g/dl)	34 (68)
Severe anemia (Hb < 8 g/dl)	9 (18)
Thrombocytopenia	
Moderate (<100.000/mcl)	11 (22)
Severe (<50.000/mcl)	7 (14)
Driver.mutations	
CALR	14 (28)
JAK2	26 (52)
MPL	6 (12)
Triple negative	4 (8)
Bone marrow fibrosis grade	
1	4 (8.2)
2	21 (42.9)
3	24 (49)
ECOG.PS	
0	18 (36)
1	29 (58)
2	3 (6)
HCT-CI	
0	12 (24)
1–2	21 (42)
>2	17 (34)
Splanchnic vein thrombosis	12 (24)
Spleen size ≥22 cm	12 (24)
Splenectomy	10 (20)
Karnofsky ≤70	3 (6)
Trasfusion.dependent.before transplant	24 (48)
Ruxolinitib o Fedratinib before transplant	26 (52)
Cytogenetic	
Favorable abnormality	4 (8.7)
Normal karyotype	29 (63)
Other abnormalities	7 (15.2)
Very high-risk abnormalities	6 (12)
Time from diagnosis to transplant (years), median (range)	2.4 (0.2–15.1)
Donor	
Sibling	10 (20)
Haploidentical	6 (12)
Matched unrelated donor	26 (52)
Mismatched unrelated donor	8 (16)
Source of stem cells	
Bone marrow	2 (4)
Peripheral blood	48 (96)

Table 1. continued

Characteristics at transplant	All, $n = 50$ (%)
Conditioning regimen	
Bu + Flu	12 (24)
Flu + Mel	5 (10)
Flu + Thiotepa	3 (6)
Thiotepa + Bu + Flu	30 (60)
ATG yes	37 (74)
Conditioning intensity	
RIC	48 (96)
MAC	2 (4)

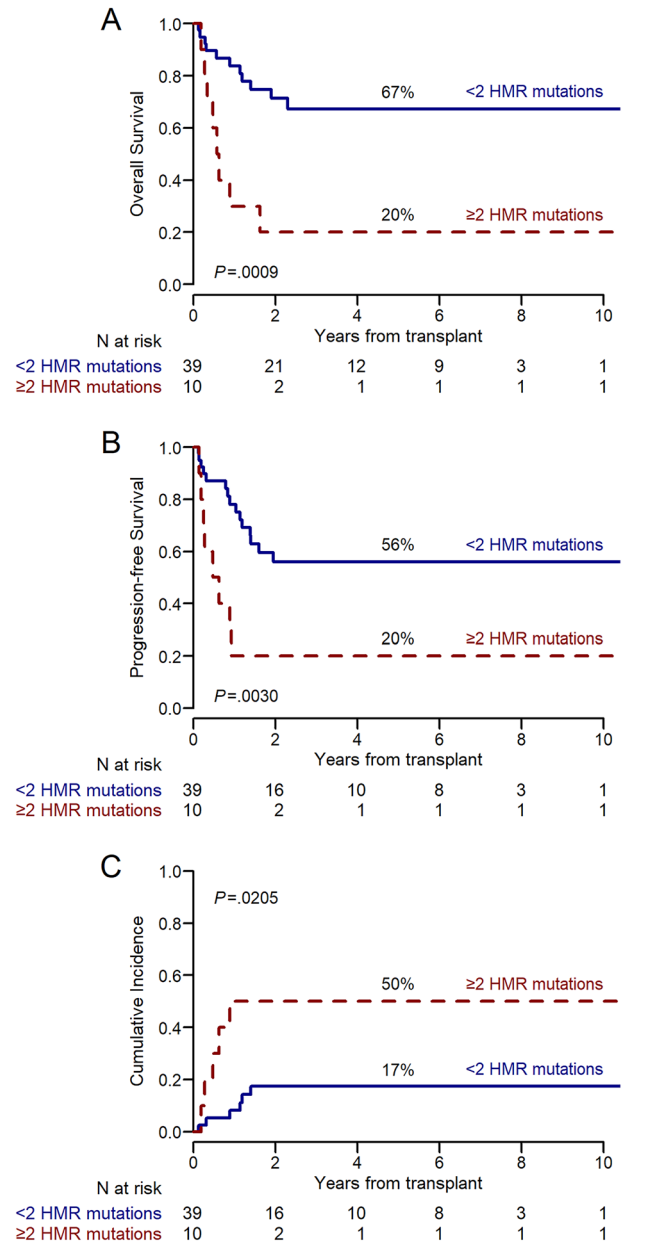

Fig. 1 Outcome of patients according to the pre-transplant number of high molecular risk mutations. Overall survival (**A**), progression-free survival (**B**), and non-relapse mortality (**C**). (Blue continous line: none or only one HMR mutation, red dotted line: 2 or more HMR mutations).

Exploring the prognostic impact of the number of HMR mutations, in the non-transplant setting, a large, retrospective study [9] showed that having two or more HMR mutations predicted the worst survival, with a median OS of 2.6 years compared to 7.0 years for one mutation and 12.3 years for no mutations. Two or more mutations were also associated with shortened leukemia-free survival. In the transplant setting, the prognostic value of the number of HMR has less evidence. Gagelmann et al. [7] investigated the impact of having more than three additional mutations beyond driver mutations but did not find an association with worse outcomes. Conversely, we found that the presence of two or more HMR mutations was associated with inferior OS, PFS, and NRM. This discrepancy could be explained by the smaller cohort size included in our study, but also by a higher prevalence of secondary MF in our study population, which may reflect different biology and post-transplant prognosis for post-PV/post-ET MF compared to primary MF.

Regarding the prognostic relevance of the single additional mutations, we confirmed, through multivariate analysis, that only *DNMT3A* mutations have prognostic value. Mutations in *DNMT3A* are associated with poor outcomes in acute myeloid leukemia [10]. In myeloproliferative diseases, *DNTM3A* mutations are widely recognized [11], but the prognostic role of *DNMT3A*, particularly in the transplant setting, requires confirmation by larger series.

Previous research suggested that *ASXL1* mutations adversely affect transplant outcome [3, 7]. Our findings, similar to a previous study [4], did not confirm an association between *ASXL1* and a worse transplant outcome. This difference could be related to factors such as the lower number of patients evaluated in our study, heterogeneity in the conditioning regimens, or a higher prevalence of *ASXL1* mutations (detected in 51% of our patients).

Data indicate that *TP53* mutations (particularly the multi-hit mutational status) [8] are associated with a poor transplant outcome. Our study confirms that *TP53* mutations are associated with a higher relapse risk after transplant, although it was not significant in multivariate analysis, and no impact on survival was observed. The limited patient number might account for this, but the data still suggest that a transplant could be curative for this challenging subset of MF patients.

The role of chimerism in predicting the transplant outcome in MF was assessed in previous studies, which all point to mixed donor chimerism as predictive of relapse [12–14]. Our results align with these findings. However, mixed donor chimerism was not predictive of decreased survival, possibly suggesting effective post-relapse strategies. A recent study on molecular monitoring after transplant [15] demonstrated that minimal residual positivity at day+30 better predicts relapse and survival than donor chimerism. Nonetheless, monitoring donor chimerism remains essential in triple-negative patients lacking other informative molecular markers.

This study has several limitations. It is retrospective and conducted at a single center, involving a heterogeneous patient population undergoing transplant with different conditioning regimens. Moreover, the relatively small sample size may also limit the statistical robustness of the findings. Larger and possibly prospective studies are needed to better understand the role of additional mutations on transplant outcome.

Despite these limitations, our findings suggest that increased molecular complexity influences transplant results. This information could aid in selecting appropriate candidates and timing for transplantation, suggesting that patients with an indication to transplant should not delay transplant until molecular or clinical progression, as acquiring new mutations may reduce the transplant benefit.

In conclusion, data from this study suggest that the molecular profile should be evaluated in all MF patients undergoing transplantation, as the complexity of the disease may affect transplant success. These insights could assist in making more informed transplant decisions in MF patients.

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DATA AVAILABILITY

Data will be made available upon reasonable request to the corresponding author.

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AUTHOR CONTRIBUTIONS

Conception and design: AR, MCF, and SS designed the clinical study. Provision of study materials or patients: MCF, FV, SS, CB, AG, AA, FL, BR, GR, GC, AC, MB, and OS. Collection and assembly of data: MCF, CP, SS, and FV. Data analysis and interpretation: CP, MCF, SS, and AR. Manuscript writing, final approval of manuscript, and accountable for all aspects of the work: all authors.

COMPETING INTERESTS
The authors declare no competing interests.

ETHICAL APPROVAL
All methods were performed in accordance with the relevant guidelines and regulations. The study was approved by the Ethics Committee at the ASST Papa Giovanni XXIII, Bergamo, under reference number 1029.

INFORMED CONSENT
Informed consent was obtained from all participants before their inclusion in the study.

ADDITIONAL INFORMATION
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