blood advances

TO THE EDITOR:

ASXL1 mutations are prognostically significant in PMF, but not MF following essential thrombocythemia or polycythemia vera

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Myelofibrosis (MF), primary (PMF) or secondary (SMF) to polycythemia vera (PPV-MF) or essential thrombocytemia (PET-MF), is characterized by a partially uncharted molecular architecture, including mutations in driver genes (*JAK2*, *CALR*, and *MPL*) and other myeloid neoplasm–associated genes.[1-3] Among these, *ASXL1* mutations (*ASXL1*^{mut}), which are associated with poor outcomes across several myeloid malignancies, are included in a category of high molecular risk (HMR) mutations in patients with PMF, along with *EZH2*^{mut}, *IDH1*^{mut}, *IDH2*^{mut}, *SRSF2*^{mut}, and *U2AF1*^{mut}.[1,4] However, a recent study[5] questioned the value of *ASXL1*^{mut} in MF and proposed a novel model, named NGS, including 4 genetic categories: *TP53*^{mut}, high risk (≥1 mutation in *EZH2*, *CBL*, *U2AF1*, *SRSF2*, *IDH1*, or *IDH2*), *ASXL1*^{mut} only, and others.

In this study, after institutional review board approval (14 560), we aimed at critically reviewing the prognostic role of *ASXL1*^{mut} with a specific focus on the distinction of PMF and SMF. We analyzed 523 patients with 2016 World Health Organization–defined MF: 330 (63%) with PMF, including 161 (49%) with prefibrotic (pre-PMF) and 169 (51%) with overt PMF, and 193 (37%) with SMF, including 85 (44%) with PPV-MF and 108 (56%) with PET-MF. Mutational analysis by targeted next-generation sequencing was performed as described[6]; details on methods are reported in the data supplement.

Median follow-up was 81 (95% confidence interval [CI], 67-93) and 77 (95% CI, 57-98) months for PMF and SMF, respectively. Patient characteristics are listed in supplemental Tables 1 to 3. Overall, 62% of patients were *JAK2*^{mut}, 24% *CALR*^{mut}, 5% *MPL*^{mut}, 8% triple negative, and 2% double mutated. *ASXL1*^{mut} were found in 157 (30%) patients, including 100 (30%) and 57 (30%) with PMF and SMF, respectively. *EZH2*^{mut} were found in 9%, *SRSF2*^{mut} in 7%, *NRAS*^{mut} in 6%, *U2AF1*^{mut} in 5%, *TP53*^{mut} and *CBL*^{mut} in 5% each, *KRAS*^{mut} in 3%, and *IDH1*^{mut} and *IDH2*^{mut} in 2% each (supplemental Tables 1-3; Figure 1A). Compared with pre-PMF, the overt PMF cohort was enriched in *ASXL1*^{mut} (41% vs 19%; $P < .0001$), *EZH2*^{mut} (17% vs 3%; $P < .0001$), *N/KRAS*^{mut} (16% vs 4%; $P = .0003$), and *U2AF1*^{mut} (8% vs 3%; $P = .0304$). HMR^{mut} were found in 54%, 24%, and 34% of patients with pre-PMF, overt PMF, and SMF, and ≥2 HMR^{mut} were found in 27%, 11%, and 10%, respectively.

In PMF, *ASXL1*^{mut} were associated with phenotypic characteristics representative of higher-risk disease, including older age (median, 64 vs 56 years; $P < .0001$), male sex (74% vs 26%; $P = .0042$), higher leukocyte count (11.9 vs 8.3 $\times 10^9$/L; $P = .0083$), lower hemoglobin level (11.2 vs 12.7 g/dL; $P < .0001$), fewer platelets (252 vs 517 $\times 10^9$/L; $P < .0001$), more peripheral blasts (1% vs 0%; $P < .0001$), bone marrow fibrosis grade ≥2 (69% vs 40%; $P < .0001$), constitutional symptoms (57% vs 34%; $P = .0001$), and transfusion dependence (43% vs 20%; $P < .0001$). *ASXL1*^{mut} clustered with *EZH2*^{mut} ($P < .0001$), *SRSF2*^{mut} ($P < .0001$), *U2AF1*^{mut} ($P = .0002$), *CBL*^{mut} ($P = .0006$), *NRAS*^{mut} ($P < .0001$), *KRAS*^{mut} ($P = .0051$), *RUNX1*^{mut} ($P = .0158$), and *SETBP1*^{mut} ($P < .0001$). In SMF, the only significant association was with *MPL*^{mut} ($P = .0207$), *EZH2*^{mut} ($P < .0001$), *U2AF1*^{mut} ($P = .0301$),

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Requests for data sharing may be submitted to Alessandro M. Vannucchi (amvannucchi@unifi.it).

The full-text version of this article contains a data supplement.

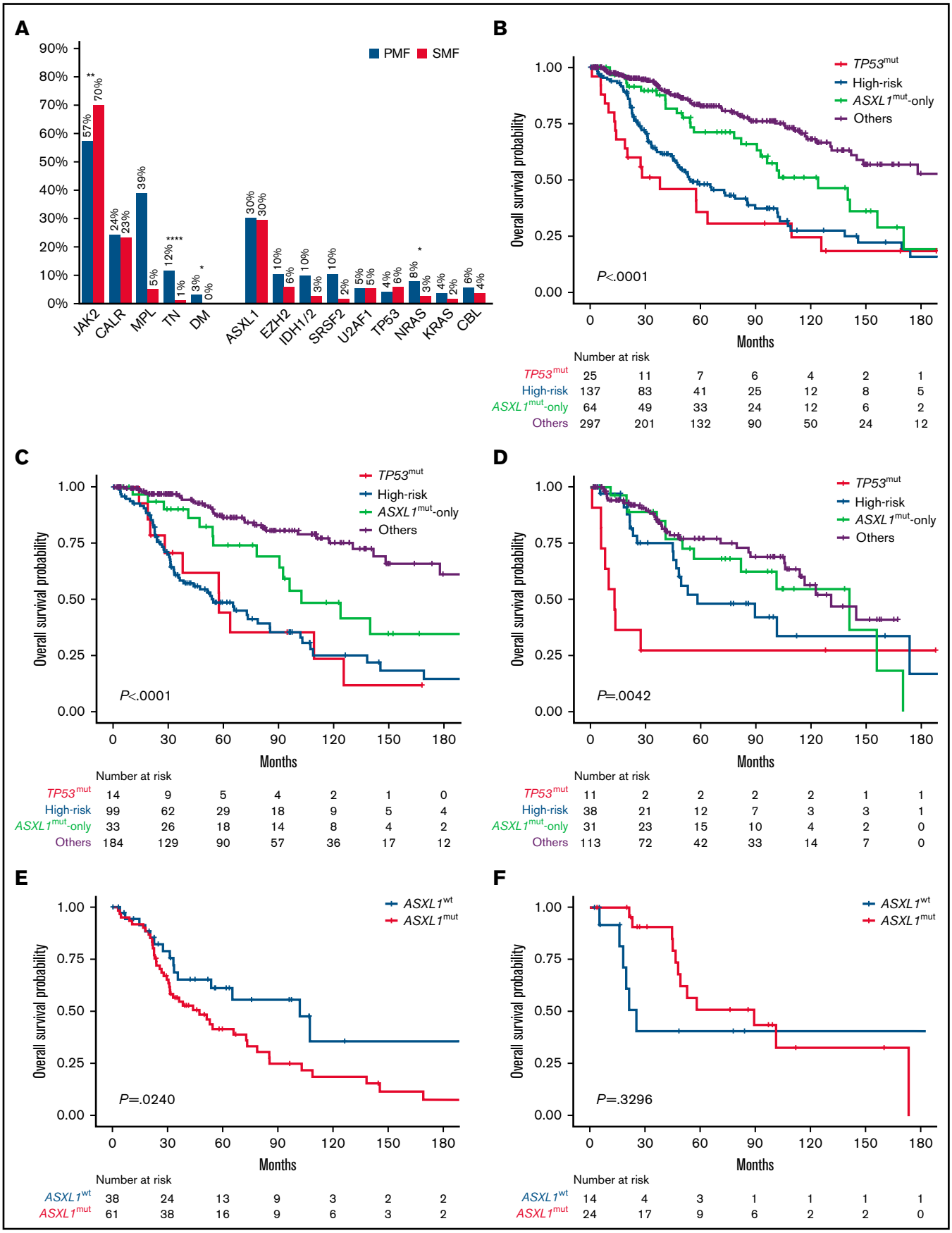

Figure 1.

and *NRAS*^{mut} ($P = .0122$). The variant allele frequency (VAF) of *ASXL1*^{mut} was higher in PMF than SMF (42% vs 26%; $P = .0129$).

According to the NGS model, patient categories were as follows: *TP53*^{mut}, 5%; high risk, 26%; *ASXL1*^{mut} only, 12%; and others, 57% (supplemental Table 1). Patients in the *TP53*^{mut} and *ASXL1*^{mut}-only categories were more likely to be diagnosed with SMF compared with those in the high-risk and others categories (44% and 48% vs 28% and 38%, respectively). The high-risk group was enriched for TN (16%), and *CALR*^{mut} were more common among those in the *ASXL1*^{mut}-only and others categories compared with *TP53*^{mut} and high-risk categories (25% and 27% vs 12% and 18%, respectively). In univariate analysis, the *TP53*^{mut} and high-risk categories had the worst OS at a median of 38 (range, 14-110) and 55 (range, 45-85) months ($P = .0039$), respectively (Figure 1B). Although remarkably better, the OS of patients in the *ASXL1*^{mut}-only group was inferior compared with that of patients in the others category (median, 124 [range, 91-156] vs 193 [range, 142 to not reached (NR)] months; $P = .0118$).

We then analyzed separately the PMF and SMF cohorts (Figure 1C-D). In PMF, the *TP53*^{mut} and high-risk categories showed the worst OS (median, 58 [range, 20-126] and 55 [range, 36-85] months, respectively), although the difference was not statistically significant, likely because of the low frequency of *TP53*^{mut} (4%; Figure 1C). Conversely, the negative prognostic impact of the *ASXL1*^{mut}-only category was magnified in comparison with the others category (median, 103 [range, 78-NR] vs 320 [range, 178-NR] months; $P = .0170$). Among patients in the high-risk group, *ASXL1*^{mut} were found in 62% and were associated with shorter OS (median, 47 [range, 31-73] vs 102 [range, 34-317] months; $P = .0240$; Figure 1E). We also noticed that median VAF was significantly higher in the *TP53*^{mut} and high-risk categories compared with the *ASXL1*^{mut}-only group in PMF (47% vs 34%; $P = .0303$), unlike SMF (27% vs 19%; $P = .128$), possibly indicating that *ASXL1*^{mut} are early driver events in PMF but might be acquired later in SMF.

In SMF, although the *TP53*^{mut} category (6%) had the worst OS (median, 13 [range, 6-NR] months), the OS of the *ASXL1*^{mut}-only category (median, 141 [range, 56-171] months) was not statistically different from those of the others (median, 131 [range, 106-NR] months; $P = .5188$) and high-risk categories (median, 58 [range, 45-174] months; $P = .3606$; Figure 1D). In the high-risk group, *ASXL1*^{mut} were found in 63% and did not influence OS (median, 90 [range, 47-174] vs 25 [range, 16-338] months; $P = .3296$; Figure 1F).

Finally, we computed the C-index, Brier score, and time-dependent area under the curve to assess the prognostic performance of standard prognostic scoring systems (Dynamic International Prognostic Scoring System [DIPSS][7] for PMF and Myelofibrosis Secondary to PV and ET–Prognostic Model [MYSEC-PM][8] for SMF) and their combinations with molecular scores (HMR and NGS; Figure 2). For this purpose, the HMR model included 3 genomic categories according to previous findings[6,9-11]: patients with no mutations in HMR genes (ie, *ASXL1*, *EZH2*, *SRSF2*, *IDH1* and *IDH2*, and *U2AF1*) and those with 1 or ≥2 HMR mutations. In PMF, the HMR-DIPSS combination was overall superior in predicting death at all time points considered (24, 48, 72, and 96 months; Figure 2A) compared with the NGS-DIPSS combination. The highest values for performance and accuracy were achieved by the Mutation-Enhanced International Prognostic Score System (MIPSS70)[6] and MIPSS70plus version 2.0.[10] In SMF, the NGS classification performed better than HMR, and its integration with MYSEC-PM achieved the highest values for performance and accuracy at all time points. Conversely, MIPSS70 and MIPSS70plus version 2.0 were largely inferior compared with other prognostic models.

Overall, these results confirm that *ASXL1*^{mut} harbor distinct phenotypic and prognostic implications in PMF and SMF. In PMF, *ASXL1*^{mut} are associated with high-risk features and a unique genetic background, unlike in SMF. To our knowledge, this is the first study reporting such a distinctive prognostic role of *ASXL1*^{mut} in PMF vs SMF. Most importantly, we confirmed that *ASXL1*^{mut}, even in the absence of any cooccurring high-risk mutations, harbor a negative prognostic impact in PMF. Accordingly, integrated clinical-molecular scoring systems, such as MIPSS70 and MIPSS70plus version 2.0, that included *ASXL1*^{mut} had the best predictive performance. It should be reinforced that these models were originally developed using series of patients with PMF only. Conversely, in SMF, the highest predictive power was achieved by the combination of MYSEC-PM and NGS variables that did not include *ASXL1*^{mut}. We acknowledge the intrinsic limitation of this study resulting from missing cytogenetic information for almost half of the patients, which prevented their inclusion in the analysis.

In summary, these findings reinforce the adverse prognostic role of *ASXL1*^{mut} in PMF and the value of current molecular integrated scores[1,6,10,11] and strengthen the contention that PMF and SMF represent 2 different biological entities, supporting the development of integrated prognostic models specific to patients with SMF.

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Contribution: P.G., G.C., and A.M.V. designed the research and analyzed data; P.G., A.M.V., F.M., G.C., G.G.L., and C.P. collected data; G.R., C. Mannarelli, and C. Maccari generated molecular data; S.R. and N.B. contributed to statistical analysis; and P.G., G.C., and A.M.V. wrote the report, which was approved by all authors.

Figure 1 (continued) Genetic mutations frequency and Kaplan-Meier estimates of overall survival. (A) Bar graph reporting the frequency of driver and nondriver genetic mutations among patients with PMF and SMF. (B-D) Kaplan-Meier estimates of overall survival (OS) in the entire series of patients with MF (B) or those with PMF (C) or SMF (D) separately, according to the 4-tier genomic classification (NGS) proposed by Luque Paz et al.[5] (E-F) Kaplan-Meier estimates of OS in high-risk patients with PMF (E) and SMF (F) by the presence or absence of *ASXL1*^{mut}. *$P < .1$, **$P < .001$, ****$P < .0001$. DM, double mutated; TN, triple negative; WT, wild type.

A

Comparison of the prognostic performance of standard prognostic scoring systems and their combinations with molecular scores

		Events at 24 months		Events at 48 months		Events at 72 months		Events at 96 months	
	C-index	Brier score	AUC	Brier score	AUC	Brier score	AUC	Brier score	AUC
PMF									
HMR	69.0	0.036	73.8	0.084	75.8	0.109	74.1	0.126	75.9
NGS	70.6	0.037	70.8	0.087	76.1	0.114	74.7	0.133	73.0
DIPSS	72.8	0.035	77.0	0.081	81.4	0.103	84.1	0.114	83.4
DIPSS+HMR	78.4	0.035	81.1	0.076	86.4	0.093	**88.1***	0.102	**87.9***
DIPSS+NGS	78.4	0.035	80.0	0.077	85.7	0.096	86.9	0.107	85.4
MIPSS70	77.8	**0.034***	83.9	**0.071***	87.3	**0.086***	87.1	**0.097***	84.9
MIPSS70+ v2.0	**78.8***	0.035	**84.0***	0.074	**87.5***	0.090	87.3	0.104	84.5
SMF									
HMR	55.5	0.055	45.3	0.100	49.3	0.137	55.1	0.161	56.6
NGS	52.1	0.054	53.5	0.097	56.7	0.134	58.8	0.157	60.4
MYSEC-PM	57.8	0.048	75.4	0.081	75.5	0.113	66.0	0.136	65.4
MYSEC-PM +HMR	**62.4***	**0.048***	73.5	0.082	75.6	0.113	69.0	0.137	68.8
MYSEC-PM +NGS	58.2	**0.048***	**76.2***	**0.080**	**77.1***	**0.112***	**69.6***	**0.135***	**69.5***
MIPSS70	55.2	0.051	74.4	0.091	64.6	0.126	62.4	0.149	62.7
MIPSS70+ v2.0	58.6	0.054	59.7	0.096	61.6	0.134	56.0	0.158	60.3

Notes: Asterisk indicate the best values.

Abbreviations: AOU: Area under the curve; DIPSS: Dynamic international prognostic scoring system; HMR: High molecular risk; MIPSS70: Mutation-enhanced international prognostic score system; MYSEC-PM: Myelofibrosis secondary to PV and ET–prognostic model; PMF: Primary myelofibrosis; SMF: Secondary myelofibrosis

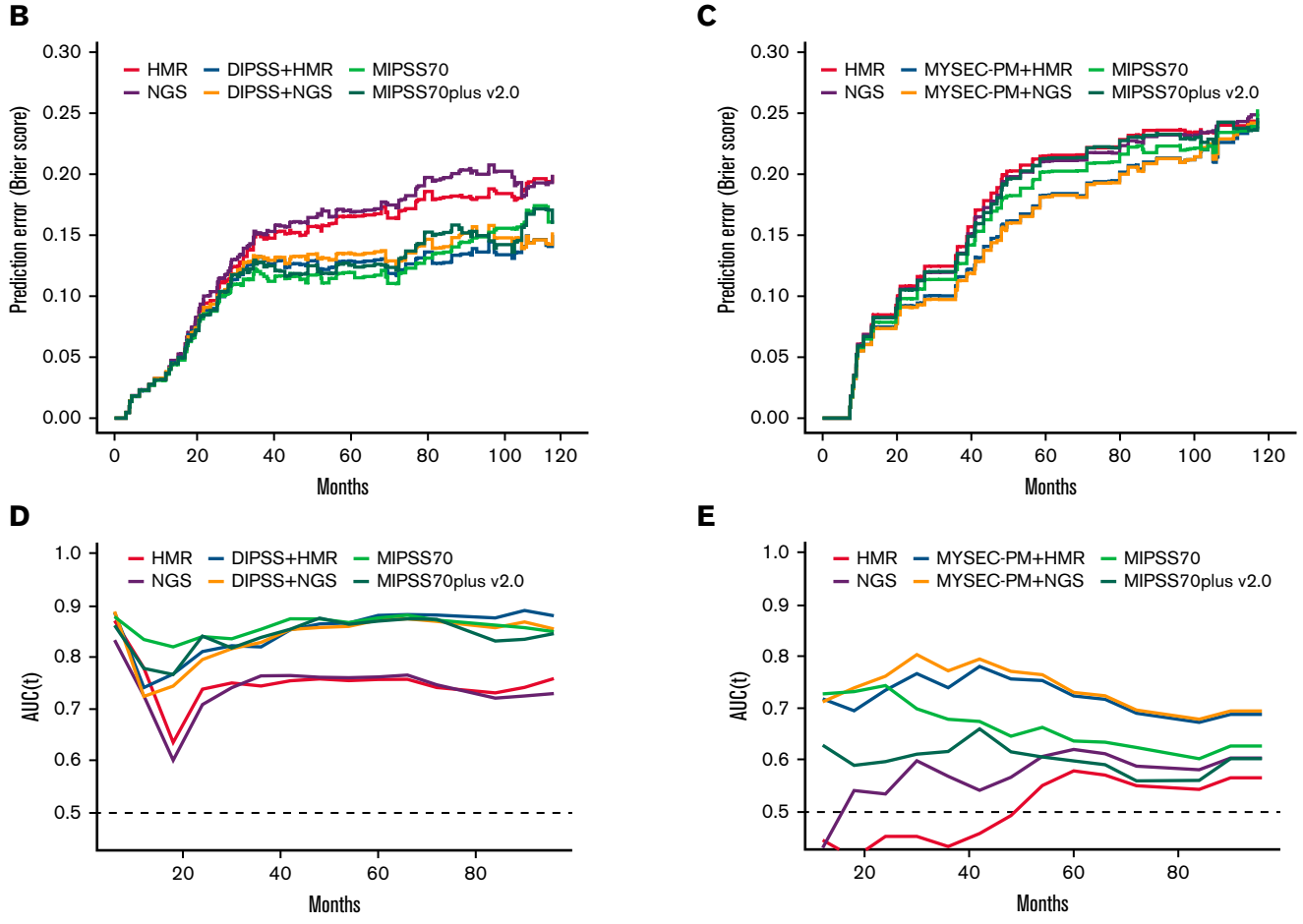

Figure 2.

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Figure 2 (continued) Performance of prognostic scoring systems. (A) Comparison of the prognostic performance among standard prognostic scoring systems (DIPSS for PMF and MYSEC-PM for SMF), their combinations with molecular scores (HMR and NGS), and novel integrated clinical-molecular score systems (MIPSS70 and MIPSS70plus version 2.0). For the purpose of the study, the HMR model included 3 genomic categories: patients with no mutations in HMR genes (ie, *ASXL1*, *EZH2*, *SRSF2*, *IDH1* and *IDH2*, and *U2AF1*), patients with 1 HMR mutation, and patients with ≥2 HMR mutations. (B-C) Brier score for prediction of death measured over time for standard and integrated prognostic scoring systems in PMF (B) and SMF (C). (D-E) Time-dependent area under the curve (AUC) for prediction of death for standard and integrated prognostic scoring systems in PMF (D) and SMF (E).