REGULAR ARTICLE

blood advances

Baseline and serial molecular profiling predicts outcomes with hypomethylating agents in myelodysplastic syndromes

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Key Points

- Baseline and serial molecular profiling by NGS can predict outcomes with HMAs in MDS patients.
- Serial molecular profiling during therapy of patients with mutant *TP53* can identify patients who may benefit from allogeneic transplantation.

Hypomethylating agents (HMAs) are widely used in the treatment of myelodysplastic syndromes (MDSs), yet identifying those patients unlikely to benefit remains challenging. We assessed response and overall survival (OS) in 247 patients molecularly profiled by next-generation sequencing (NGS) before first-line HMA therapy, and a subset of 108 patients were sequenced serially during treatment. The most common mutations included *TP53* (33.1%), *ASXL1* (19%), *TET2* (16.5%), *DNMT3A* (14.1%), and *SRSF2* (12.1%). The overall response rate was 42.1%, with the composite *TET2*-mutant/*ASXL1* wild-type genotype representing the strongest predictor of response (overall response rate, 62.1%; complete remission rate, 34.5%). The median OS for the cohort was 15 months, and the number of mutations detected by NGS (hazard ratio [HR], 1.22; $P = .02$), as well as mutations in *TP53* (HR, 2.33; $P = .001$) and *EZH2* (HR, 2.41; $P = .04$) were identified as independent covariates associated with inferior OS in multivariable analysis. Serial molecular profiling revealed that clearance of *TP53* mutations during HMA therapy was associated with superior OS (HR, 0.28; $P = .001$) and improved outcome in patients proceeding to allogeneic hematopoietic cell transplantation. These data support baseline molecular profiling by NGS in MDS patients treated with HMAs and provide novel observations of sequential profiling during therapy that provide particular value in *TP53*-mutated disease.

Introduction

Myelodysplastic syndromes (MDSs) display hematologic and prognostic heterogeneity, which illustrates the need for accurate risk stratification and individualized therapy. In patients with higher-risk disease, the hypomethylating agents (HMAs) azacitidine and decitabine are the standard first-line treatment owing to their clinical activity and the potential to extend overall survival (OS).[1-3] However, despite their widespread adoption, fewer than 50% of patients respond with poor outcomes observed after treatment failure, which highlights the need for a reliable strategy to identify patients most likely to benefit from therapy.[4,5]

In up to 90% of MDS patients, recent advances in next-generation sequencing (NGS) have identified recurrent somatic mutations in genes that function in RNA splicing, epigenetic regulation, gene transcription, and cell signaling.[6-11] These mutations underlie key pathogenic features of MDS and, in addition to classic clinical and cytogenetic features, independently impact OS.[6,7,12] It has been hypothesized that somatic mutations may also serve as biomarkers predictive for response to HMA therapy, but studies to date have been inconsistent. Mutations in the *TET2* gene, a dioxygenase involved in DNA demethylation, were linked to a higher rate of response to HMAs in several studies,[13-15] but in

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other studies, they had no impact.[16-18] One such study showed that improved response rates in patients with a *TET2* mutation were largely dependent on concurrent *ASXL1* genotype.[13] Mutations in the vital tumor suppressor gene *TP53* are consistently associated with inferior survival; however, the impact of *TP53* mutations on response to HMA therapy has been inconsistent.[12,13,18-20] The adverse effect of *TP53* mutations on treatment outcome also extends to allogeneic hematopoietic cell transplantation (allo-HCT), in which only a small subset of patients achieve long-term remissions.[21-23]

Sequential molecular profiling of disease during active treatment has been investigated as a means to characterize clonal evolution.[11] Falconi et al[18] found that although allelic frequencies in the majority of genes do not change, *TP53*-mutant clone size was reduced with HMA treatment, whereas clones remained detectable in all patients evaluated. Nonetheless, the value of serial molecular profiling of patients to help direct treatment strategies remains largely unexplored.[24] Notably, we recently reported that achievement of NGS negativity was a powerful independent predictor of OS in MDS and in patients with secondary acute myeloid leukemia (AML).[25]

Heterogeneity and sample size have been limitations in previous studies that aimed to identify genetic covariates predictive for outcome with HMA therapy. Because of the widespread commercial availability of NGS, molecular profiling is increasingly used, so its utility in the clinical setting requires further delineation. Herein, we report on molecular predictors of outcome in one of the largest reported cohorts to date of predominantly high-risk MDS patients treated with HMA therapy. Baseline molecular profiling before treatment was required for study entry, with sequential profiling during treatment performed in a subset of patients.

Patients and methods

Patient selection and inclusion

Patients treated at the H. Lee Moffitt Cancer Center and Research Institute between 2010 and 2019 were retrospectively analyzed. Patients with a World Health Organization (WHO)–defined diagnosis of MDS, myelodysplastic syndrome/myeloproliferative neoplasm (MDS/MPN) overlap syndrome, or AML (with 20% to 30% bone marrow myeloblasts) were included. All patients received first-line therapy with an HMA, which included azacitidine, decitabine, or azacitidine in combination with an additional agent. NGS was performed within 6 months before the first cycle of HMA therapy (median time from NGS to day 1 of therapy was 17 days; maximum time was 170 days). Clinical characteristics were abstracted from multiple timepoints, including the date of diagnosis, at initiation of HMA therapy (timepoint used for analyses involving baseline predictors of response and OS), and at the time of response to HMA. Patients proceeding to allo-HCT after HMA therapy were included. This study was approved by the Institutional Review Board of the H. Lee Moffitt Cancer Center and Research Institute.

Mutational profiling

All patients underwent molecular profiling by NGS with sequencing performed on DNA extracted from peripheral blood or bone marrow mononuclear cells. The NGS panels targeted up to 406 genes, and each gene that was included in statistical analyses (supplemental Table 1) was sequenced in a minimum of 92% of patients. A minimum variant allele frequency (VAF) of 5% was used to call single nucleotide variants, and a cutoff of 10% was used for insertions or deletions. Each reported mutation was further evaluated by the study team and referenced in databases including Catalogue Of Somatic Mutations In Cancer (COSMIC) and dbSNP to ensure that reported mutations were both somatic and pathogenic. Sequential molecular profiling was evaluated in a subset of patients who had NGS testing performed during or at the completion of HMA therapy (before any subsequent therapy or allo-HCT).

Definitions of response and survival

Response to HMA therapy was assessed by using the International Working Group 2006 response criteria for MDS.[26] Comparisons for evaluating predictors of response to HMA therapy assessed both the overall response rate (ORR) and the complete remission (CR) rate. ORR was defined as patients achieving CR, marrow CR (mCR), partial remission (PR), or hematologic improvement (HI). OS was defined as the duration from first day of HMA therapy until death, with surviving patients censored at time of last patient contact.

Statistical methods

Categorical variables were compared using Fisher's exact and χ^2 tests, and quantitative data were compared using the Mann-Whitney *U* test and Wilcoxon rank sum tests. The Kaplan-Meier method was used to estimate OS and the log-rank test was used to compare OS between groups in univariable analyses. Patients were not censored at the time of allo-HCT in reported analyses. A binary logistic regression model was used in multivariable analyses to determine covariates for response. Multivariable analysis of OS used the Cox proportional hazards model, with clinical and molecular variables having a P value $< .10$ in univariable analysis included in the model. The backward elimination method was used to select variables for the final multivariable model, and variables with a P value $> .05$ were excluded. Patients sequenced on targeted panels that did not include a specific gene of interest were excluded from all analyses evaluating that gene. Statistical analyses were performed using GraphPad Prism version 8.3 and IBM SPSS statistics version 27.

Results

Baseline characteristics of the cohort

A total of 247 patients were included in the cohort, and baseline characteristics are summarized in Table 1. The median age at diagnosis was 69 years (range, 24-89 years) and 64.7% of the patients were male. The majority of patients (79.1%) were classified at diagnosis as higher-risk (intermediate, high, or very high risk) by using the Revised International Prognostic Scoring System (IPSS-R). Among 51 patients classified as lower risk at diagnosis, 31 (60.8%) progressed to higher-risk disease before treatment with HMA was initiated; 90.3% of patients were classified as higher risk by IPSS-R at the time of HMA therapy. The most common subtypes by WHO classification at diagnosis were MDS with excess blasts-2 (25.5%) and MDS with excess blasts-1 (23.9%), and 184 patients (74.5%) had ≥5% blasts at treatment initiation. The diagnosis was classified as therapy-related in 24.3% of patients.

All 247 patients were treated with an HMA in the first-line setting, and azacitidine was the treatment used for 81% of the patients. A total of 29 patients received decitabine as first-line HMA therapy:

Table 1. Baseline characteristics of the cohort at time of diagnosis

Baseline characteristic	Total cohort (N = 247)
Median age (range), y	69 (24-89)
Percent male	64.7
IPSS-R risk stratification	
Very low	15 (6.1)
Low	36 (14.8)
Intermediate	57 (23.4)
High	51 (20.9)
Very high	85 (34.8)
WHO classification	
MDS-SLD	11 (4.4)
MDS-MLD	52 (21.1)
MDS-RS-SLD	2 (0.8)
MDS-RS-MLD	14 (5.7)
MDS with isolated del(5q)	2 (0.8)
MDS-EB1	59 (23.9)
MDS-EB2	63 (25.5)
MDS-U	7 (2.8)
MDS/MPN	16 (6.5)
AML (20%-30% blasts)	21 (8.5)
Therapy-related myeloid neoplasm	60 (24.3)
AML transformation	81 (35.8)
First-line HMA therapy	
Azacitidine	200 (81)
Decitabine	29 (11.7)
HMA plus additional agent	18 (7.3)
Proceeded to allo-HCT	61 (24.7)
Patients with detectable mutation on NGS	213 (86.2)
Median no. of mutated genes (range)	2 (0-8)
Median absolute neutrophil count (range), $\times 10^9$/L	1.16 (0.06-28.8)
Median hemoglobin (range), g/dL	9 (6.8-13.6)
Median platelet count (range), $\times 10^9$/L	64.5 (8-1073)
Median bone marrow blast percentage (range)	8 (0-30)

Data are presented as n (%) unless otherwise specified.

MDS/MPN, myelodysplastic syndrome/myeloproliferative neoplasm; MDS-EB1, MDS with excess blasts-1; MDS-EB2, MDS with excess blasts-2; MDS-MLD, MDS with multilineage dysplasia; MDS-RS-MLD, MDS with ring sideroblasts and multilineage dysplasia; MDS-RS-SLD, MDS with ring sideroblasts and single lineage dysplasia; MDS-SLD, MDS with single lineage dysplasia; MDS-U, MDS unclassifiable.

6 received a 10-day regimen, and 18 (7.3%) received azacitidine in combination with an additional agent. Those agents included investigational therapies (n = 9; no patients treated with eprenetapopt [APR-246] or magrolimab were included), lenalidomide (n = 5), checkpoint inhibitors (n = 3), and venetoclax (n = 1). The median number of cycles of HMA therapy was 4, and the median time from diagnosis to therapy initiation was 1.6 months.

Spectrum of somatic mutations

A somatic mutation was identified in at least 1 gene in 213 patients (86.2%), with a median of 2 mutated genes (range, 0-8 mutated genes). The observed frequency of mutations is detailed in Figure 1; *TP53* (33.1%), *ASXL1* (19%), *TET2* (16.5%), *DNMT3A* (14.1%), and *SRSF2* (12.1%) were the most commonly mutated genes.

A complex karyotype was observed in 87 patients, and 71 (81.6%) harbored a mutation in *TP53*. Likewise, 86.6% of all *TP53*-mutated patients had a complex karyotype. *TP53* was the sole mutation identified in 46 patients (56.1%) who had a *TP53* mutation. In 63 patients (76.8%), there was a single mutation in *TP53*, 18 (22%) carried 2 mutations, and 1 (1.2%) harbored 3 mutations. Biallelic loss of *TP53* (defined by patients with >1 *TP53* mutation, *TP53* mutation and a chromosome 17 abnormality, or a *TP53* VAF >70%) was seen in 51 patients (62.2%). Among 47 *ASXL1*-mutated patients, 36 (76.6%) harbored frameshift or nonsense mutations.

Covariates for response to HMA therapy

In the total cohort, 37 patients achieved CR as best response (15%), 34 mCR, 4 PR, and 29 HI for an ORR (CR + mCR + PR + HI) of 42.1%. In univariable analysis, no clinical variables evaluated at the time of HMA initiation were predictive of ORR; however, an absolute neutrophil count <1000 cells per μL ($P = .002$), number of cytopenias ($P = .03$), and increased marrow blast percentage ($P = .02$) were associated with a higher CR frequency. No clinical variables were predictive of ORR or CR in multivariable analyses.

Molecular covariates for response are summarized in Table 2. In univariable analysis, the *TET2*-mutant/*ASXL1*-wild-type (WT) genotype was predictive of both CR (34.5% vs 12.1%; $P = .004$) and ORR (62.1% vs 39.8%; $P = .03$), and *ASXL1* mutations (4.3% vs WT, 17.5%; $P = .02$) and spliceosome mutations (7.8% vs WT, 18.4%; $P = .048$) were associated with inferior rates of CR. In multivariable analyses (accounting for absolute neutrophil count, number of cytopenias, bone marrow blast percentage, WHO classification, age, sex, and genotype of *TET2*, *ASXL1*, *TP53*, and spliceosome genes), *TET2* mutations ($P = .03$) and the *TET2*-mutant/*ASXL1*-WT genotype ($P = .02$) remained statistically significant covariates for CR. No specific somatic mutations or molecular patterns remained statistically significant for ORR in multivariable analysis; however, a trend was observed for the *TET2*-mutant/*ASXL1*-WT genotype ($P = .10$), *TET2* mutations ($P = .07$), and a lower ORR with *ASXL1* mutations ($P = .08$). On the basis of these findings, response rates were stratified and compared across combinations of *TET2* and *ASXL1* genotypes as depicted in Figure 2.

Responses were compared between therapeutic agents with an ORR of 40.4% for azacitidine regimens compared with 55.2% for decitabine ($P = .16$) and CR rates of 13.3% and 27.6% ($P = .05$), respectively. Identical comparisons were made in *TP53*-mutated patients with an ORR of 40.6% vs 55.6% ($P = .29$) and CR rates of 17.2% vs 33.3% ($P = .19$) with azacitidine compared with decitabine, respectively.

Patterns of HMA failure were investigated in a subset of patients (n = 106) that identified *TP53* as the most common somatic mutation in patients who progressed to AML during or at completion of therapy (13 of 24 patients), and 37.1% of *TP53*-mutated patients exhibited progression. In contrast, progression to AML was rare in patients with spliceosome mutations: 1 instance occurred in an *SRSF2*-mutated patient (n = 12), and no instances were observed among *SF3B1*- or *U2AF1*-mutated patients.

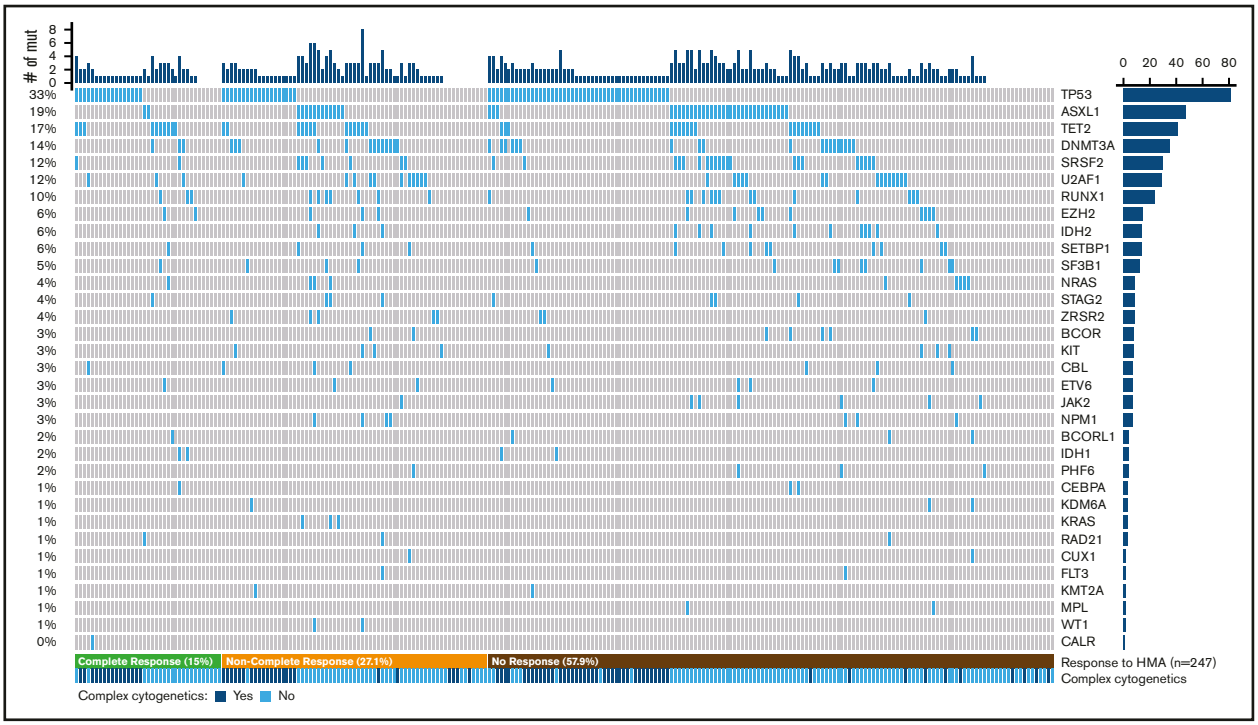

Figure 1. Spectrum of mutations identified in the cohort. Co-mutation plot showing mutations (mut) observed in the cohort; each column represents an individual patient. Mutations are listed in descending order by frequency observed, with the frequency of each mutation on the left and the total number of patients on the right. Patients are organized by response to HMA therapy (bottom), and the presence of complex cytogenetics is listed.

Covariates for OS

The median OS of HMA-treated patients was 15 months (95% confidence interval, 12.4-17.5 months). OS was significantly longer in patients responding to HMA therapy (18.5 vs 12.8 months in nonresponders; HR, 0.60; $P = .009$). Among responders, there was no difference in OS observed in patients achieving CR vs HI (15.6 vs 21.7 months; HR, 0.74; $P = .46$), whereas patients with mCR exhibited an OS comparable to that of nonresponders (12.2 vs 12.8 months; HR, 0.93; $P = .78$). Additional clinical variables and their impact on OS are summarized in supplemental Table 2. Only IPSS-R category ($P < .001$), response to HMA (HR, 0.30; $P < .001$), and allo-HCT (HR, 0.28; $P < .001$) retained independent significance in the final multivariable model.

Analysis of somatic mutations and their impact on survival is summarized in Table 3. In univariable analysis, mutations in *EZH2* (Figure 3A), *TP53* (Figure 3B), the absence of detectable mutations by NGS (Figure 3C), and the number of mutated genes were associated with OS. Stratifying patients into groups based upon the total number of mutated genes was highly predictive in *TP53*-WT patients, with cut points of 0 vs 1 to 3 vs 4+ mutations most informative (Figure 3D). In multivariable analysis accounting for molecular (Table 3) and clinical (supplemental Table 2) variables, the number of somatic mutations ($P = .02$) and mutations in *TP53* (HR, 2.33; $P = .001$) and *EZH2* (HR, 2.41; $P = .04$) retained significance. Complex karyotype was also associated with inferior OS (10.2 vs 25.8 months in those without a complex karyotype; HR, 3.26; $P < .001$), which remained significant in multivariable analysis when replacing *TP53* mutations ($P = .001$). Among *TP53*-mutated patients, there was no significant difference in OS between patients with monoallelic vs biallelic loss (10.2 vs 9.6 months; HR, 0.82; $P = .5$).

Analysis of OS according to specific HMA (ie, azacitidine vs decitabine) showed similar outcomes within the entire cohort (15.5 vs 12.3 months; HR, 0.72; $P = .30$) and among *TP53*-mutated patients (7.8 vs 12.3 months; HR, 1.21; $P = .62$). Similarly, there was no difference in OS among *TP53*-mutated patients treated with 5-day (n = 12) vs 10-day (n = 6) decitabine regimens (12.3 vs 7 months; HR, 0.33; $P = .08$).

Sequential molecular profiling

Sequential molecular profiling was performed in 108 patients (43.7%) either during or at completion of HMA therapy, among whom 12 had no mutation detectable at baseline. Sequential NGS was performed on bone marrow samples in 89 patients (82.4%) and peripheral blood samples in 19 patients (17.6%), with a median time to sequential NGS of 4.5 months. Among 96 patients with a detectable mutation at baseline, 25 (26%) had clearance of all mutations with HMA therapy; these patients exhibited a median OS of 15.6 months compared with 14.2 months in those with mutation persistence (HR, 0.62; $P = .27$). Findings were similar when not accounting for clearance of the clonal hematopoiesis-associated genes *DNMT3A*, *TET2*, and *ASXL1* (HR, 0.76; $P = .48$), and clearance was observed in 29 patients (30.5%). Fifty patients (52.1%) had clearance of at least 1 mutation (median OS, 15.6 vs 14.2 months in those without clearance of at least 1 mutation; HR, 0.77; $P = .45$), and 33 patients (30.6%) acquired at least 1 new mutation (median OS, 21.7 vs 14.2 months in those who did not acquire at least 1 new mutation; HR, 0.93; $P = .83$) during therapy.

Table 2. Molecular predictors of response

Genotype	No. of patients	CR rate, %	Univariable *P*	Multivariable OR (95% CI)	Multivariable *P*	ORR rate, %	Univariable *P*	Multivariable OR (95% CI)	Multivariable *P*
Total cohort	247	15				42.1			
NGS result			.4				.22		
No mutation	28	21.4				53.6			
≥1 mutation	213	14.6				40.8			
TET2			.09	2.80 (1.09-7.24)	.03*		.08	1.92 (0.93-3.94)	.07
Mut	41	24.3				56.1			
WT	188	12.8				40.4			
ASXL1			.02*	0.13 (0.02-1.08)	.06		.07	0.49 (0.22-1.08)	.08
Mut	47	4.3				29.8			
WT	200	17.5				45.0			
***TET2*-mut/*ASXL1*-WT**			.004*	3.15 (1.20-8.30)	.02*		.03*	1.99 (0.86-4.59)	.10
Present	29	34.5				62.1			
Other	206	12.1				39.8			
DNMT3A			.31				.85		
Mut	35	8.6				45.7			
WT	193	16.1				43.0			
EZH2			>.99				.59		
Mut	15	13.3				33.3			
WT	232	15.1				42.7			
DNA methylation mutation			.7				.33		
Mut	79	16.5				48.1			
WT	148	14.2				41.2			
Epigenetic regulation			.47				.36		
Mut	120	13.3				40			
WT	115	17.4				46.1			
SF3B1			.7				.4		
Mut	13	7.7				30.8			
WT	217	15.7				44.2			
Any spliceosome			.048*	0.46 (0.16-1.33)	.15		.57	0.86 (0.46-1.63)	.65
Mut	77	7.8				40.3			
WT	152	18.4				44.7			
RUNX1			>.99				>.99		
Mut	24	12.5				41.7			
WT	223	15.2				42.2			
Signaling pathway			.18				.57		
Mut	32	6.3				37.5			
WT	196	16.3				44.4			
TP53			.08	1.29 (0.56-3.0)	.55		.79	1.01 (0.54-1.89)	.96
Mut	82	20.7				43.9			
WT	165	12.1				41.2			

Response rates among the total cohort stratified by genotype; multivariable analysis was performed using a binary logistic-regression model (ORR defined as CR + mCR + PR + HI).
CI, confidence interval; OR, odds ratio.
*Denotes statistical significance ($P < .05$).

At completion of HMA therapy, acquisition of new mutations was identified in 26.7% of HMA nonresponders, 43.2% of HMA responders who subsequently progressed, and 47.1% of patients who progressed to AML.

Similar analyses were performed among patients harboring *TP53* mutations (n = 47), with 15 patients (31.9%) clearing all baseline mutations (median OS, 13.3 vs 10.3 months with persistent mutations; HR, 0.46; $P = .06$). When solely analyzing clearance

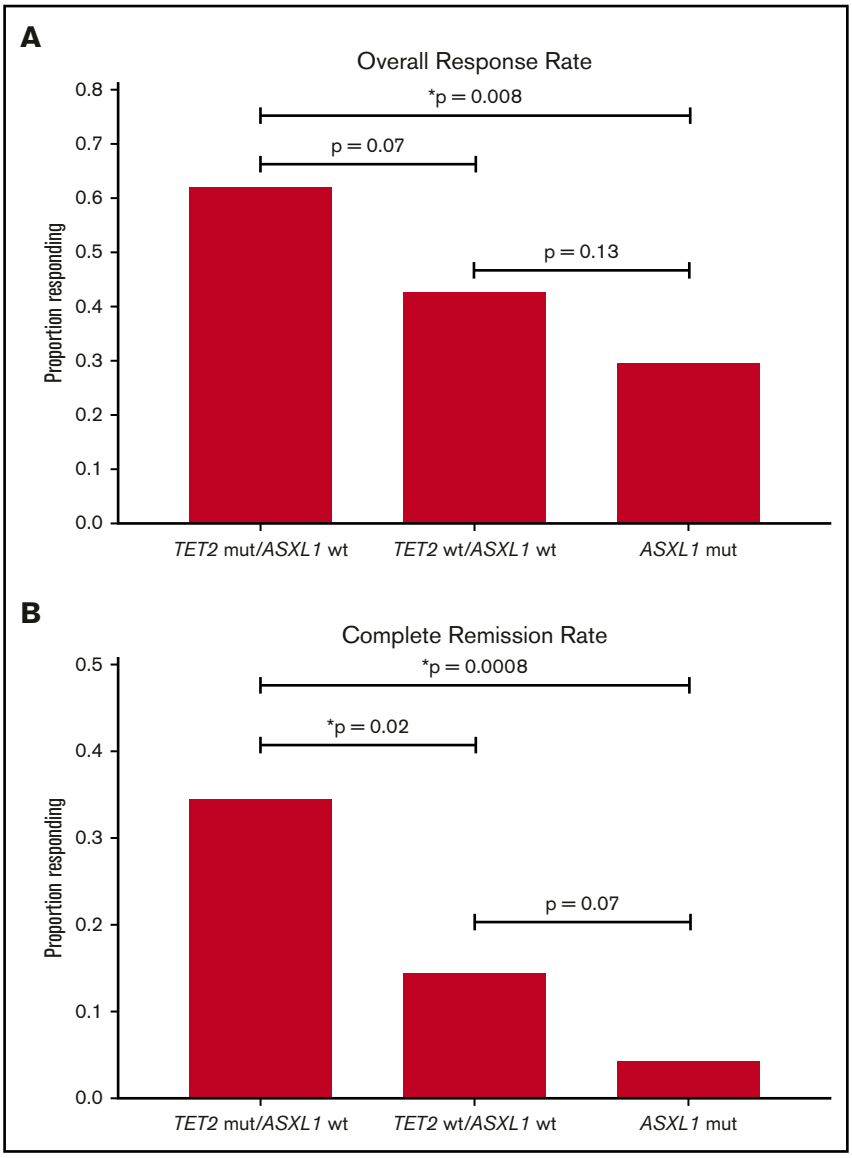

Figure 2. Response stratified by *TET2* and *ASXL1* genotype. Comparison of overall response rate (A) and complete remission rate (B) among combinations of *TET2* and *ASXL1* genotypes (*TET2*-mutated [mut]/*ASXL1*-wt [wild-type], n = 29; *TET2*-wt/*ASXL1*-wt, n = 159; *ASXL1*-mut, n = 47). The vertical axis represents the frequency of response and comparisons between specific groups are annotated. *Denotes statistical significance ($P < .05$).

of *TP53*, this was predictive of an improvement in median OS (15.6 vs 7.7 months with *TP53* persistence; HR, 0.28; $P = .001$; Figure 4A) among the 22 patients (46.8%) exhibiting *TP53* clearance with HMA therapy. Of these 22 patients, 12 (54.5%) achieved CR, 3 (13.6%) mCR, 3 (13.6%) HI, and 4 (18.2%) stable disease, with no difference in OS between patients who achieved CR and those who did not (median OS, 14.5 vs 13.1 months; HR, 0.68; $P = .61$). Patients with clearance of *TP53* mutations demonstrated a lower median VAF (12% vs 33.3%; $P = .02$) but no difference in frequency of biallelic loss ($P > .99$), associated chromosome 17 abnormalities ($P > .99$), class of mutation ($P = .36$), or complex cytogenetics ($P = .47$). In addition, there was no difference in time to sequential NGS analysis (3.7 vs 4.1 months; $P = .68$).

Within the total cohort, patients who proceeded to allo-HCT (n = 61) after first-line treatment demonstrated improved OS compared with those treated with HMA alone (not reached vs 12.4 months; HR, 0.35; $P < .0001$; Figure 4B). Among these patients, 27 underwent serial molecular analysis before allo-HCT; 10 patients cleared all detectable mutations (median OS not reached vs 14.4 months with detectable mutations; HR, 0.47; $P = .47$; supplemental Figure 1). Among 16 *TP53*-mutated patients proceeding to allo-HCT, 7 achieved a complete molecular remission before transplantation with a trend toward improved OS compared with patients with clonal persistence (median OS, 25.2 vs 11.7 months; HR, 0.41; $P = .10$; Figure 4C). Furthermore, patients with *TP53* clearance before allo-HCT had a survival benefit compared with patients treated solely with an HMA (25.2 vs 7.7 months; HR, 0.28; $P = .005$; Figure 4D), but patients with *TP53* persistence did not (11.7 vs 7.7 months; HR, 0.60; $P = .16$; Figure 4E). Patients demonstrating *TP53* clearance before allo-HCT were younger than those with *TP53* persistence (median, 53 vs 67 years old, respectively; $P = .09$), but time to serial molecular analysis was similar (median, 4.1 vs 4.0 months; $P = .56$).

Discussion

Treatment of higher-risk MDS with HMAs remains the standard of care, but the rate of nonresponse is high, and putative biomarkers

Table 3. Molecular predictors of OS

Genotype	No. of patients	Median OS, mo	Univariable analysis		Multivariable analysis		
			HR	*P*	HR	95% CI	*P*
Total cohort	247	15					
NGS result			0.46	.02*	1.34	0.54-3.33	.52
No mutation	28	24					
≥1 mutation	213	14.1					
No. of mutations				.005*	1.22	1.03-1.44	.02*
TET2			0.89	.67			
Mut	41	16.1					
WT	188	14.4					
ASXL1			0.84	.49			
Mut	47	18.6					
WT	200	14.4					
***TET2*-mut/*ASXL1*-wt and *TP53*-wt**			0.51	.13			
Present	21	16.1					
Other	218	14.4					
DNMT3A			1.37	.24			
Mut	35	11.4					
WT	193	15.5					
EZH2			2.83	<.001*	2.41	1.03-5.64	.04*
Mut	15	9.9					
WT	232	16.1					
DNA methylation mutation			0.96	.83			
Mut	79	14.2					
WT	148	14.4					
Epigenetic regulation mutation			1.04	.82			
Mut	120	14.2					
WT	115	15.5					
SF3B1			0.77	.57			
Mut	13	18.5					
WT	217	14.4					
Any spliceosome			0.68	.07	1.06	0.58-1.95	.84
Mut	77	19.1					
WT	152	13.1					
RUNX1			1.09	.78†			
Mut	24	12.4					
WT	223	15.5					
Signaling pathway			1.15	.61			
Mut	32	14.4					
WT	196	14.5					
TP53			2.82	<.001*	2.33	1.41-3.85	.001*
Mut	82	9.7					
WT	165	21.7					

HR, hazard ratio.
*Denotes statistical significance ($P < .05$).
†Associated with a statistically significant ($P < .001$) impact on OS when stratifying by both *RUNX1* and *TP53* genotype (see supplemental Figure 2).

for treatment benefit show inconsistent results across studies. Furthermore, the clinical impact of monitoring mutations longitudinally during therapy remains largely unexplored. To this end, we present results from the largest single-institution cohort to date of higher-risk MDS patients treated with HMA therapy after baseline molecular profiling by NGS. We identify several NGS-based

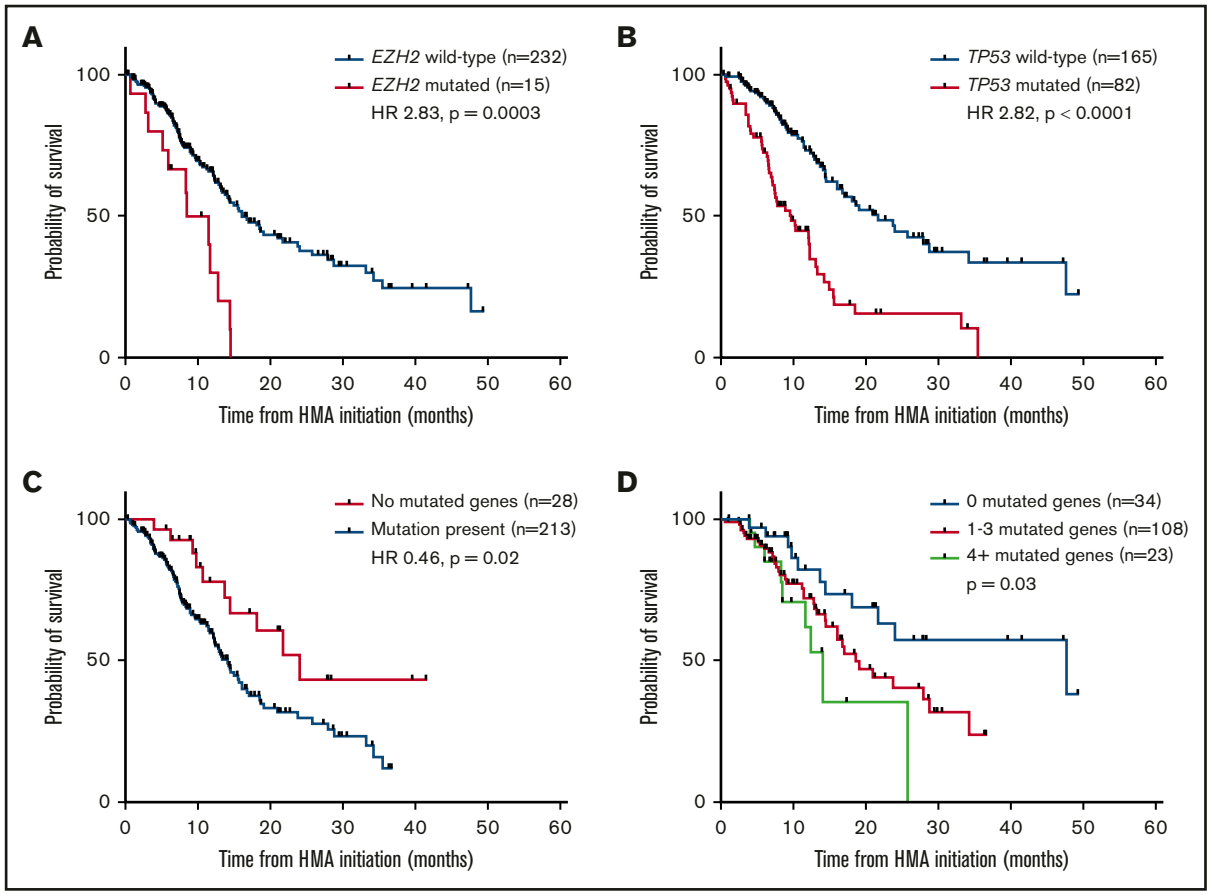

Figure 3. OS of specific molecular predictors. Survival impact of *EZH2* mutations (A), *TP53* mutations (B), and the absence of detectable mutations (C) on NGS. (D) Survival stratified by number of mutations in *TP53*-wt patients.

molecular covariates for response and survival with novel observations from a large subset analyzed serially during therapy.

Mutations in *TET2* and *ASXL1* genes emerged as the most informative variables associated with response to HMA therapy in multivariable analysis that accounted for both clinical and molecular variables. Although *TET2*-mutated patients as a whole exhibited ORR and CR rates higher than those of the overall cohort, this benefit was largely driven by patients harboring the *TET2*-mutant/*ASXL1*-WT genotype, confirming previous observations by Bejar et al[13] from a smaller cohort. Notably, *ASXL1* mutations were the strongest molecular covariate for inferior response, in particular CR, and they largely negated the benefit of *TET2* mutations. Similar outcomes were observed in patients with dual *TET2* and *ASXL1* mutations compared with *TET2*-WT/*ASXL1*-mutated patients.

Mutation of a spliceosome component gene was the only other molecular covariate associated with response in univariable analysis, which demonstrated a lower rate of CR but no difference in ORR. Nonetheless, multivariable analysis by logistic regression revealed no impact of spliceosome mutations and identified no baseline clinical variables that retained significance. Although *DNMT3A* mutations were initially hypothesized to predict for response to HMAs, this study aligns with more recent reports that have not identified such a benefit, which provides further definitive evidence that no such association exists.[13,15,16,18,27,28] Thus, stratifying by composite *TET2*/*ASXL1* genotype seems to be the strongest molecular biomarker for response to HMA therapy, outperforming all other clinical and molecular variables evaluated. As in previous studies, improved response rates in *TET2*-mutated patients did not correlate with a survival advantage, although a trend toward improved OS was found in *TET2*-mutated patients who had both *ASXL1* and *TP53* WT.[13,14]

The median OS of the total cohort was 15 months, considerably lower than that observed in the AZA-001 study but comparable to that reported in similar real-world post-marketing data for higher-risk MDS patients treated with HMA.[1,29-31] Patients achieving a response to an HMA experienced an improvement in OS, with benefit largely driven by patients achieving CR or HI. Patients who achieved mCR as best response had no evidence of a survival benefit and their outcomes were nearly identical to those of nonresponders, thus raising the question of the value of including mCR in response assessments in future studies.

An improvement in OS was observed in patients who proceeded to allo-HCT after first-line HMA therapy. This benefit was restricted to patients with ≥5% blasts at treatment initiation, but it was independent of response to HMA. Importantly, the survival advantage with allo-HCT was maintained in multivariable analysis that accounted for both clinical and molecular variables, which supports the use of HMA in the pretransplant setting.

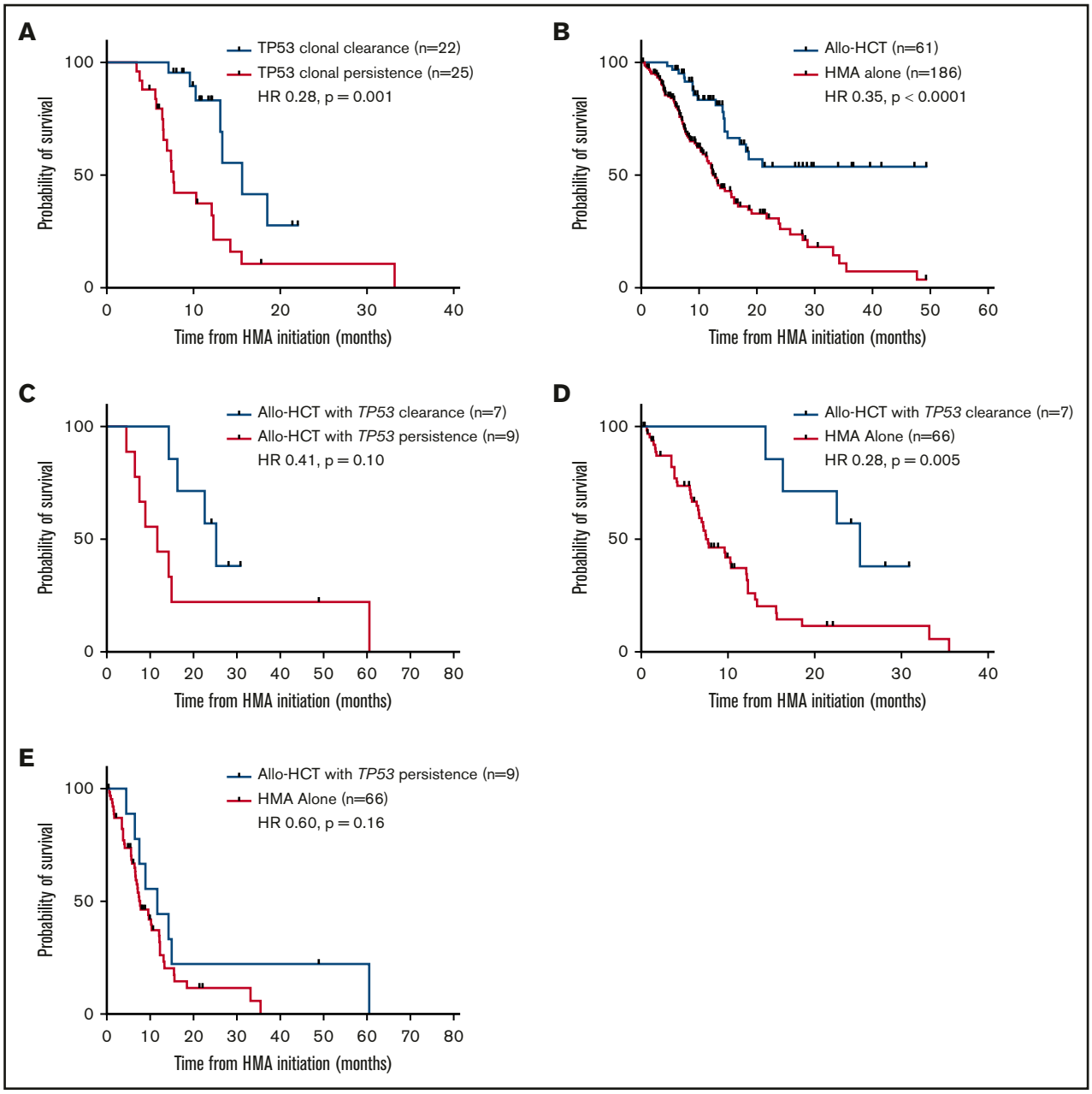

Figure 4. Impact of *TP53* clonal clearance on OS with HMA therapy and allo-HCT. (A) Impact of *TP53* clonal clearance on OS in all HMA-treated patients undergoing sequential molecular profiling (median OS, 15.6 months with *TP53* clearance and 7.7 months with *TP53* persistence). (B) OS of all patients within the cohort proceeding to allo-HCT after first-line therapy with an HMA. (C) Impact of *TP53* clonal clearance on OS among patients proceeding to allo-HCT (median OS, 25.2 vs 11.7 months, respectively). Comparison of OS among patients proceeding to allo-HCT vs patients receiving HMA therapy alone in those with *TP53* clonal clearance before transplant (25.2 vs 7.7 months) (D) vs those with *TP53* clonal persistence (11.7 vs 7.7 months) (E).

Molecular covariates for inferior OS in univariable analysis included the total number of mutations and mutations in the *TP53* and *EZH2* genes, whereas the absence of mutations was associated with improved OS. Each of the molecular categories predictive of inferior OS retained independent significance in the final multivariable model. Previous work has yielded conflicting survival data on the impact of *ASXL1* mutations and HMA therapy.[12,32,33] In this study, *ASXL1* mutations had no impact on OS despite inferior response rates, whereas *EZH2* mutations were associated with a poor OS, which is contrary to previous observations.

Interestingly, genotypes that were associated with inferior OS (ie, *TP53*- and *EZH2*-mutated) were not associated with primary resistance to HMAs; *TP53*-mutated patients actually had a trend toward higher CR rates. Likewise, genotypes associated with primary resistance to HMAs (ie, *TET2*-WT/*ASXL1*-mutated) had no direct impact on OS and were largely mutually exclusive with those

that did, suggesting distinct mechanisms of primary vs secondary resistance.

Our findings are in agreement with previous reports that HMA therapy does not overcome the adverse prognostic impact of *TP53* mutations, which remain perhaps the strongest predictor of inferior outcomes.[13,19,32] *TP53* was the most commonly mutated gene in this study (33%), which is a considerably higher frequency than that observed in larger genomic studies of MDS.[6,7] However, a higher frequency has been reported in cohorts from tertiary cancer centers, which likely reflects referral bias.[13,19,34,35] Importantly, this represents the largest cohort to date of *TP53*-mutated patients treated with first-line HMA and provides important historical data for ongoing trials evaluating novel agents in combination with HMA, including eprenetapopt and magrolimab, in this molecular subgroup.

To account for a potential adverse prognostic impact related to the high frequency of *TP53* mutations, patients with *TP53*-WT were analyzed as a separate cohort for both response rate (supplemental Table 3) and OS (supplemental Table 4). A trend toward improved OS was seen in spliceosome-mutated patients overall, but this seemed to be driven by the majority of *TP53*-mutated patients who fall within the spliceosome WT group. No survival impact was seen in the *TP53*-WT cohort or in multivariable analysis of the total cohort. On the contrary, no difference was observed when solely evaluating *RUNX1* genotypes, but stratifying by both *RUNX1* and *TP53* status did delineate 3 groups (supplemental Figure 2); *RUNX1*-mutated patients had an intermediate OS closer to that of *TP53*-mutated patients (median OS, 12.4 vs 9.7 months for *TP53*; HR, 0.59; $P = .10$). In further evaluating the impact of mutational burden, stratifying patients into specific groups based on the number of mutations was less predictive in the total cohort. This seemed to be largely a result of the inferior impact of *TP53* mutations which were associated with a lower number of coexisting mutations, thus impacting the survival of patients with 1 or 2 mutations. When isolating the *TP53*-WT cohort, however, patients with 1 to 3 mutations demonstrated similar outcomes, and grouping patients into categories of those with 0, 1 to 3, or 4 or more mutations was predictive of OS.

In addition to assessing baseline somatic mutations before initiation of therapy, 108 patients had serial sequencing performed during or at the completion of therapy, primarily from bone marrow samples. The number of each individual mutation was relatively low in this subgroup, so analyses instead focused on gain or clearance of any mutation. Of particular interest, complete clearance of all mutations, clearance of at least 1 mutation, or the gain of 1 or more mutations had no impact on survival. These findings are in line with investigations in AML showing that preleukemic mutations commonly observed in clonal hematopoiesis and MDS (including *DNMT3A*, *TET2*, and *ASXL1)* are significantly less likely to clear with therapy, with persistence having no impact on outcome.[36,37]

Falconi et al[18] previously reported that while the VAF of the majority of somatic mutations remained stable during HMA therapy, *TP53* mutations were the exception, and a reduction in mutational burden was routinely observed. In this study, nearly half the evaluable patients achieved *TP53* clearance by NGS (VAF $<5\%$), and these patients demonstrated a significant improvement in OS. Interestingly, mutation clearance was seen in the majority of patients with CR (12 of 15 patients), but nearly half of such patients had a response less than CR, including 4 patients with stable disease. Stratifying by quality of response had no further impact on OS, suggesting that *TP53* clearance supersedes clinical response in prognostic importance in *TP53*-mutated patients.

Similarly, clearance of *TP53* mutations also portended for improved survival after allo-HCT, albeit in a small sample size. These findings align with those reported by Welch et al[20] in *TP53*-mutated MDS and AML patients treated with a 10-day regimen of decitabine. Although further validation in larger cohorts is necessary, these findings support a novel strategy for selecting *TP53*-mutated patients as allo-HCT candidates. Despite improved outcomes, however, just 3 of the 16 *TP53*-mutated patients undergoing allo-HCT experienced a relapse-free survival lasting longer than 24 months, which supports the need for new therapeutic strategies in this group.

Patient heterogeneity is a potential limitation of the study. The majority of the cohort consisted of patients with higher-risk MDS, but small numbers of patients with lower-risk MDS, MDS/MPN, and AML (20% to 30% blasts) were included. However, this is consistent with typical populations treated with HMAs in clinical practice. To account for any bias related to diagnosis, WHO category was included in multivariable analyses and demonstrated no impact on outcome. Similarly, variability in the timing of sequential NGS analysis has the potential to bias these results, although the median time to sequential NGS was 4.5 months, consistent with expected timing of response assessments in patients treated with HMA.

In conclusion, this study expands on previous work that evaluated molecular biomarkers for outcome with HMA therapy and supports the incorporation of baseline molecular profiling before treatment. It also provides further insight into the application of serial sequencing during treatment, which seems particularly valuable in *TP53*-mutated patients. These findings suggest that somatic mutations and classic clinical variables do not fully account for variability in outcomes, with novel biomarkers yet to be discovered.

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Authorship

Contribution: A.M.H. and D.A.S. conceived of and designed the study; A.M.H., R.S.K., N.A.A., O.C., D.A.S., J.S., and M.H. collected and assembled the data; A.M.H., R.S.K., and D.A.S. analyzed and interpreted the data; and A.M.H., R.S.K., N.A.A., O.C., S.Y., E.P., J.S., M.H., C.T., K.L.S., J.E.L., A.F.L, and D.A.S. wrote the manuscript.

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