Hypomethylating agents are associated with high rates of hematologic toxicity in patients with secondary myeloid neoplasms developing after acquired aplastic anemia

Most patients with acquired aplastic anemia (AA) treated with immunosuppressive therapy (IST) develop clonal hematopoiesis (CH) in recovering hematopoietic stem and progenitor cells (HSPC).[1-3] Up to 20% of patients go on to develop secondary myelodysplastic syndrome (MDS) or acute myeloid leukemia (AML).[4,5] Despite an improved understanding of CH and risk factors for post-AA myeloid neoplasms (MN),[1,3,4,6-8] optimal management of patients with post-AA MN remains unknown.

Curative treatment for patients with high-risk MN typically requires allogeneic stem cell transplantation (SCT). Hypomethylating agents (HMA) prolong survival in MDS patients and are commonly administered before SCT for disease control. HMA are given as outpatient therapy and have a favorable toxicity profile.[9,10] Because AA patients have a depletion of their HSPC, we hypothesized that post-AA MN patients may be more susceptible to hematologic toxicities with HMA therapy. We thus evaluated treatment outcomes and treatment-emergent adverse events (TEAE) in post-AA MN patients treated at the University of Pennsylvania (Penn) and Children's Hospital of Philadelphia (CHOP) between 2005 and 2022.

Patients were identified retrospectively through ICD-9/10 codes and the Penn/CHOP Comprehensive Bone Marrow Failure (BMF) registry, followed by chart review as a part of Institutional Review Board-approved research. MN was defined according to World Health Organization (WHO) criteria. AA diagnosis was established using standard criteria,[11] requiring systematic exclusion of other causes including transient cytopenias, neoplasia, and inherited BMF. TEAE were graded according to the Common Terminology Criteria for Adverse Events (CTCAE, version 5.0). Overall survival (OS) from MN diagnosis to death was estimated using the Kaplan-Meier method. Each post-AA patient who received at least one cycle of HMA was randomly matched to three similarly-aged non-AA MDS patients who received at least one cycle of HMA at Penn for MDS during the study period. Endpoints of interest included incidence of febrile neutropenia; grade 3-4 infections and hemorrhage; intracranial hemorrhage (ICH); duration of grade 4 neutropenia, anemia, and thrombocytopenia; hospitalizations; HMA cycle delays; dose reductions; therapy discontinuation; receipt of SCT; and death during HMA treatment. Treatment cycles were 28 days from the start of HMA, with delay defined as >14 days from planned subsequent cycle initiation.

The study cohort included 14 post-AA MN patients who were diagnosed with AA at a median age of 49 years (range, 1-71) (Table 1). Thirteen of 14 received IST with anti-thymocyte globulin and cyclosporine. One had hepatitis-associated AA that was initially managed with growth factors and transfusions followed by recovery of blood counts after development of paroxysmal nocturnal hemoglobinuria. Six patients (43%) had at least one AA relapse, with two (14%) developing MN within 1 year of IST retreatment. Median time from AA to MN transformation was 5 years (range, 0.25-30), with a median age at MN of 56.5 years (range, 5-75). After MN diagnosis, patients were followed for 33 months (range, 1-107).

No patients had evidence of dysplasia at AA diagnosis, and of those with evaluable cytogenetic (N=11) and somatic sequencing (N=4) data, none had clonal abnormalities at AA diagnosis. After IST, three developed CH without morphologic dysplasia (*Online Supplementary Figure S1*). At MN diagnosis, nine patients (64%) had cytogenetic abnormalities, most commonly monosomy 7 (36%). Five (36%) had poor-or very-poor-risk cytogenetics by Revised International Prognostic Scoring System (IPSS-R) criteria. Eight (57%) had somatic mutations classically associated with secondary malignancy in AA,[6] most commonly *ASXL1* (29%) and *RUNX1* (21%). By WHO criteria, eleven patients were classified as having MDS, one AML, and two clonal cytopenia of undetermined significance (CCUS; 1 had transfusion-dependent thrombocytopenia with *ASXL1*, *RUNX1*, and *DNMT3A* mutations, another 1 had der(3;15) with 3q gain).

Patients with post-AA MN were treated according to institutional standard practices for pediatric and adult patients. Three pediatric patients were treated with SCT. In contrast, ten of 11 adults received HMA, administered to induce hematologic response, prevent leukemic progression, and reduce disease burden. One adult died before receiving treatment. Nine MDS/CCUS patients were treated with azacitidine, while the AML patient received azacitidine and venetoclax. Overall survival was 71% (95% confidence interval [CI]: 51-99) at 1 year and 56% (95% CI: 34-90) at 3 years. OS was not associated with age at diagnosis (hazard ratio [HR]=1.01; *P*=0.637) or premalignant CH (HR=0.63; *P*=0.67). Nine patients (3 pediatric, 6 adult) received SCT (*Online Supplementary Table S1*) - seven (78%) were alive and six (66%) remained in remission at time of analysis (Figure 1A). Two adults (22%) relapsed following SCT. One pediatric patient had primary graft failure with high-titer HLA alloimmunization and died after second SCT. All six patients transplanted after HMA had residual dysplasia, including two with ≥5% marrow blasts at SCT. Five of six stopped HMA due to toxicity. Me-

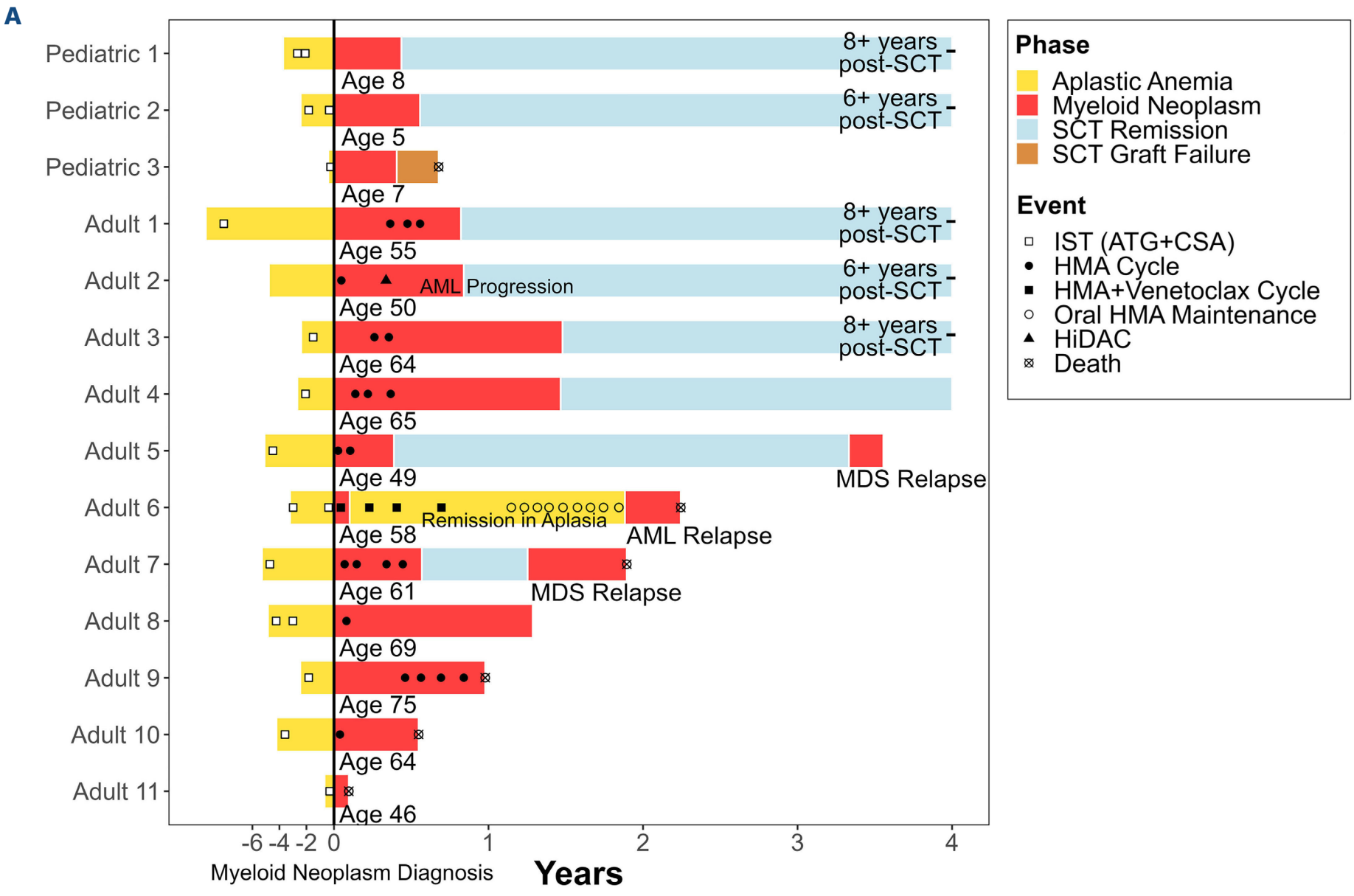
Table 1. Baseline characteristics of investigated patients.

Characteristics of the 14 pediatric and adult patients with post-AA myeloid neoplasms			
Characteristic	**Value**		
Total patients, N	14		
Median age in years at AA diagnosis (range)	49 (1-71)		
Median age in years at MN diagnosis (range)	56.5 (5-75)		
Median time in years from AA to MN diagnosis (range)	5 (0.25-30)		
Female sex, N (%)	6 (43)		
Initial AA therapy, N (%)			
ATG + CSA	10 (71)		
ATG + CSA + eltrombopag	1 (7)		
CSA + androgen	1 (7)		
ATG + androgen	1 (7)		
Growth factor support	1 (7)		
Patients with at least 1 AA relapse, N (%)	6 (43)		
AA status at MN diagnosis, N (%)			
Complete remission, off immunosuppression	7 (50)		
At least partial remission, on immunosuppression or growth factor	5 (36)		
Refractory, on immunosuppression	2 (14)		
Presence of PNH clone†, N (%)	3 (21)		
Characteristics of post-AA adults treated with HMA compared to non-AA MDS patients			
Characteristic	**Post-AA MN, N=10**	**Non-AA MDS, N=30**	***P***
Median age in years at MN diagnosis (range)	62.5 (49-75)	62 (46-75)	0.656
Female sex, N (%)	5 (50)	10 (33)	0.457
Diagnosis, N (%)			
AML	1 (10)	0	
MDS	8 (80)	30 (100)	
CCUS	1 (10)	0	
IPSS-R, N (%)			0.507
Very high	1 (10	8 (27)	
High	2 (20)	8 (27)	
Intermediate	4 (40)	9 (30)	
Low or very low	2 (20)	2 (7)	
Unknown	1 (10)	3 (10)	
IPSS-M, N (%)			0.723
Very high	2 (20)	4 (13)	
High	1 (10)	10 (33)	
Moderate high or moderate low	3 (30)	5 (17)	
Low or very low	1 (0)	3 (10)	
Unknown	3 (30)	8 (27)	
Cellularity at MN diagnosis, %, median (range)	55 (30-65)	65 (10-95)	0.365
Blasts at MN diagnosis, %, median (range)	3.5 (0-24)	5 (0-20)	0.115
ANC x10^9/L prior to HMA, median (range)	1.31 (0.04-5.80)	1.50 (0.03-10.50)	0.092
Hemoglobin g/dL prior to HMA, median (range)	10.3 (7.2-13.0)	9.6 (6.2-16.4)	0.055
Platelets x10^9/L prior to HMA, median (range)	36.5 (10-255)	115 (6-477)	0.007
Patients who received SCT after HMA, N (%)	6 (60)	15 (50)	0.721
Months from MN diagnosis to SCT, median (range)	8.5 (4-21), N=6	5 (4-15), N=15	0.452
Months last chemotherapy to SCT, median (range)	4 (1-12), N=6	1 (1-3), N=15	0.171
Marrow blasts at SCT, %, median (range)	4 (0-32), N=6	1 (0-5), N=15	0.084

†Two patients had clinically significant hemolytic PNH, and 1 had a subclinical PNH clone. AA: acquired aplastic anemia; MN: myeloid neoplasm; ATG: anti-thymocyte globulin; CSA: cyclosporine; PNH: paroxysmal nocturnal hemoglobinuria; AML: acute myeloid leukemia; MDS: myelodysplastic syndrome; CCUS: clonal cytopenia of undetermined significance; IPSS-R: Revised International Prognostic Scoring System; IPSS-M: Molecular International Prognostic Scoring System; ANC: absolute neutrophil count; HMA: hypomethylating agent; SCT: stem cell transplant.

dian time from MN diagnosis to SCT was 6 months (range, 3-21). Four patients who did not undergo SCT after HMA either died (N=2) or could no longer receive SCT after TEAE (N=2). Median OS was 12 months in patients who did not receive SCT (N=5) and not reached in transplanted patients (Figure 1B).

Ten post-AA MN adult patients treated with HMA were then included in comparative analysis of TEAE, with outcomes



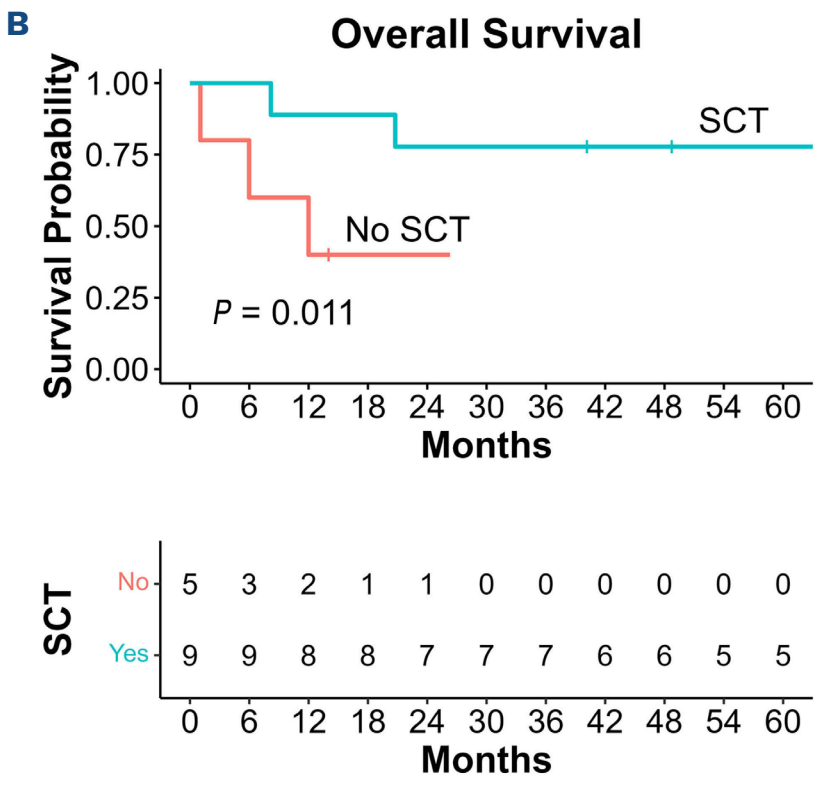


Figure 1. Clinical courses of 14 post-aplastic anemia patients with secondary myeloid neoplasms. Swimmer plot (A) depicting clinical courses; year 0 on the x-axis is diagnosis of myeloid neoplasm with negative time representing time from aplastic anemia diagnosis with scale compressed given large range (0.25-30 years); and overall survival (B) of post-aplastic anemia patients plotted from the time of diagnosis of myeloid neoplasia. AML: acute myeloid leukemia; CSA: cyclosporine; HMA: hypomethylating agent; HiDAC: high-dose cytarabine; IST: immunosuppressive therapy; ATG: anti-thymocyte globulin; MDS: myelodysplastic syndrome; SCT: allogeneic stem cell transplant.

compared to 30 randomly-selected, aged-matched MDS patients without antecedent AA. The cohorts had similar baseline characteristics (Table 1) aside from lower median platelet counts (36x10^9/L *vs*. 115x10^9/L; *P*=0.007) in post-AA patients.

Post-AA patients, most of whom were in remission from AA when they developed MN, experienced significantly higher rates of hematologic toxicities with HMA administration (Table 2). Per cycle, post-AA patients experienced longer grade 4 neutropenia (median 9 *vs*. 1.5 days; *P*=0.044) and grade 4 thrombocytopenia (median 13 *vs*. 0 days; *P*=0.003), more febrile neutropenia (80% *vs*. 17%, RR 4.8; *P*<0.001), grade 3-4 infections (90% *vs*. 13%, RR 6.8; *P*<0.001), and grade 3-4 hemorrhages (40% *vs*. 7%, RR 6.0; *P*=0.026). Two post-AA patients (20%) experienced ICH on HMA *versus* none in the non-AA cohort. Post-AA patients had more hospital admissions - 18 in 25 total chemotherapy cycles (72%) compared to 12 in 207 cycles (6%) in the matched cohort (RR 12.4; *P*<0.001). Post-AA MN patients had more treatment delays (28% *vs*. 8% of cycles, RR 3.4; *P*=0.007), more HMA discontinuation (70% *vs*. 3%, RR 21.0; *P*<0.001), and received fewer cycles of HMA (median 2.5 *vs*. 6; *P*=0.021). Death occurred following TEAE in 20% of post-AA MN patients *versus* no deaths on HMA in the matched cohort (RR 14.1; *P*=0.06). Median time from MN diagnosis to SCT was 8.5 months (range, 4-21) in post-AA *versus* 5 months (range, 4-15) in non-AA patients. Three-year OS in the post-AA MN cohort was shorter (57% *vs*. 88%), though this was not statistically significant (*P*=0.18). Three-year OS in post-AA *versus* non-AA patients who underwent SCT was similar (83% *vs*. 92%; *P*=0.6). Exclusion of the one post-AA AML patient did not affect the results (*Online Supplementary Table S2*).

Prior registry-based studies evaluated outcomes of post-AA MN patients after SCT, with comparable OS for post-AA *versus de novo* MDS, and better OS compared to other types of secondary MDS.[12-14] However, these analyses were restricted to transplanted patients and did not include data on pretransplant patient management. Cytotoxic chemotherapy has been linked to increased rates of hematologic and extra-hematopoietic toxicities in inherited BMF. Ours is the first report, however, of increased toxicity in patients with a history of acquired AA. Though HMA has a tolerable toxicity profile in MDS, post-AA patients in our cohort experienced dramatically higher rates of serious infections, hemorrhage, hospitalizations, and treatment delays compared to age-matched non-AA MDS patients. Higher TEAE rates occurred with fewer HMA cycles (median 2.5 *vs*. 6) reflecting a high incidence of treatment termination. In our study, post-AA MN patients who had received multiple IST cycles for relapsed AA had particularly severe cytopenias with HMA, likely reflecting a cumulative effect of HSPC depletion by successive episodes of immune-mediated aplasia. Prospective studies are needed to develop risk stratification for post-AA MDS patients. Traditional risk models used to aid treatment in MDS such as IPSS-R and Molecular IPSS (IPSS-M) may be less predictive in post-AA MDS. AA patients treated with IST frequently have residual cytopenias and CH without overt MN transformation, and some alterations (e.g., mutations in *BCOR/BCORL1* and del(13q)) are favorable in AA patients compared to their prognostic significance in

Table 2. Comparative analysis of hematologic toxicities and treatment-emergent adverse events in ten adult post-aplastic anemia myeloid neoplasm patients and 30 non-aplastic anemia myelodysplastic syndrome controls treated with hypomethylating agents.

Hematologic parameters and HMA cycles			
Parameter	**Post-AA MN, N=10**	**Non-AA MDS, N=30**	***P***
Days per cycle ANC <0.5x10^9/L, N, median (range)	9 (0-40)	1.5 (0-33.5)	0.044
Days per cycle Hgb <8.0 g/dL, N, median (range)	5.5 (0-21)	4 (0-21)	0.270
Days per cycle platelets <25x10^9/L, N, median (range)	13 (0-35)	0 (0-21)	0.003
Total number of cycles HMA, median (range)	2.5 (1-13)	6 (2-41)	0.021
Treatment-emergent adverse events			
Adverse Event	**Post-AA MN, N=10**	**Non-AA MDS, N=30**	**Risk ratio (*P*)**
Patients with grade 3-4 bleeding events, N (%)	4 (40)	2 (7)	6.0 (0.026)
Patients with intracranial bleeding events, N (%)	2 (20)	0 (0)	14.1 (0.058)
Patients with grade 3-4 infections, N (%)	9 (90)	4 (13)	6.8 (<0.001)
Patients with febrile neutropenia, N (%)	8 (80)	5 (17)	4.8 (<0.001)
TEAE leading to dose reduction, N (%)	4 (40)	4 (13)	3.0 (0.089)
Cycles delayed >2 weeks, N (%)	7 (28), N=25 cycles	17 (8), N=207 cycles	3.4 (0.007)
Patients stopping treatment due to toxicity, N (%)	7 (70)	1 (3)	21 (<0.001)
Hospital admissions, N (% of cycles requiring hospitalization)	18 (72)	12 (6)	12.4 (<0.001)
Patients with TEAE leading to delay in SCT, N (%)	2 (20)	0	14.1 (0.058)
Patients who died from TEAE, N (%)	2 (20)	0	14.1 (0.058)

P values of <0.05 are statistically significant. HMA: hypomethylating agent; AA: acquired aplastic anemia; MN: myeloid neoplasm; MDS: myelodysplastic syndrome; ANC: absolute neutrophil count; Hgb: hemoglobin; TEAE: treatment-emergent adverse events; SCT: hematopoietic stem cell transplant.

MDS.[3,6,15] Until such data are available, we recommend an individualized approach to guide treatment decisions that considers age at AA diagnosis, hematologic parameters at recovery from IST, relapsed or refractory AA, and presence of high-risk features such as *ASXL1* or *RUNX1* mutations,[6] monosom,[7] or excess blasts.[4]

While our cohort was small, the differences in TEAE compared to age-matched non-AA MDS controls were large and statistically significant. Our data suggest that post-AA MN patients may not derive the expected benefits from HMA due to high treatment toxicity and inability to tolerate repeated chemotherapy cycles. Early SCT may be a more suitable treatment strategy in appropriate candidates. New approaches are needed to minimize toxicity and improve access to SCT in transplant-eligible post-AA MN patients.

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Contributions

MC and DB designed the study, analyzed data, and wrote the manuscript. MC, NP, and DB performed the chart review. MC, NF, SG, EH, XJB, CL, AL, SL, AM, SM, AP, DP, KP, and DB contributed clinical care for adult patients, and JO and TO for pediatric patients. TO and DB oversaw the Penn/CHOP BMF patient registry. AZ performed the bioinformatics analysis in electronic medical records. All authors contributed to manuscript revisions.

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Data-sharing statement

Data supporting the findings of this study are available from the corresponding authors upon reasonable request.

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