Genetic landscape and clinical outcomes of patients with *BCOR* mutated myeloid neoplasms

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Abstract

The *BCL6-corepressor* (*BCOR*) is a tumor-suppressor gene located on the short arm of chromosome X. Data are limited regarding factors predicting survival in *BCOR*-mutated (m*BCOR*) acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS). We evaluated 138 patients with m*BCOR* myeloid disorders, of which 36 (26.1%) had AML and 63 (45.6%) had MDS. Sixty-six (47.8%) patients had a normal karyotype while 18 (13%) patients had complex karyotype. *BCOR*-mutated MDS/AML were highly associated with *RUNX1* and *U2AF1* co-mutations. In contrast, *TP53* mutation was infrequently seen with m*BCOR* MDS. Patients with an isolated *BCOR* mutation had similar survival compared to those with high-risk co-mutations by European LeukemiaNet (ELN) 2022 criteria (median OS 1.16 *vs*. 1.27 years, *P*=0.46). Complex karyotype adversely impacted survival among m*BCOR* AML/MDS (HR 4.12, *P*<0.001), while allogeneic stem cell transplant (alloSCT) improved survival (HR 0.38, *P*=0.04). However, *RUNX1* co-mutation was associated with an increased risk of post-alloSCT relapse (HR 88.0, *P*=0.02), whereas melphalan-based conditioning was associated with a decreased relapse risk (HR 0.02, *P*=0.01). We conclude that m*BCOR* is a high-risk feature across MDS/AML, and that alloSCT improves survival in this population.

Introduction

The *BCL6-corepressor* (*BCOR*) is a tumor-suppressor gene located at the 11.4 locus of short arm of chromosome X.[1] The *BCOR* gene product is predominantly involved in suppressing myeloid regulatory genes and promotes lymphopoiesis.[1] Grossman *et al*. first demonstrated the association of somatic *BCOR* mutations with acute myeloid leukemia (AML).[2] The recent 2022 European LeukemiaNet (ELN) recommendations on acute myeloid leukemia classifies *BCOR*-associated AML in the adverse risk category.[3] However, the clinical outcomes of *BCOR*-mutated (m*BCOR*) AML has primarily been explored in pooled cohorts evaluating multiple other genes. For instance, Papaemmanuil *et al*. evaluated clinical outcomes of patients with AML stratified by genetic subgroups.[4] The study included m*BCOR* AML within the chromatin-spliceosome group, which also included patients with mutations in *SRSF2*, *SF3B1*, *U2AF1, ZRSR2, ASXL1, STAG2, BCOR, MLL*^{PTD}, *EZH2*, and *PHF6*. The study showed adverse outcomes of patients in this mutational group. Similarly, Gardin *et al*. evaluated the outcomes of a cohort of 471 patients with secondary AML (sAML)-like gene mutations which included 38 patients with m*BCOR* AML, and a trend towards worse overall survival (OS) in the presence of sAML-like mutations (HR 1.22, *P*=0.07) was observed.[5] The outcomes of patients with m*BCOR* AML were not specifically evaluated in either of the studies.
Damm *et al*. evaluated 15 patients with m*BCOR* myelodysplastic syndrome (MDS) and demonstrated an inferior OS compared to patients with wild-type *BCOR* (HR 3.3, 95% CI 1.4-8.1, *P*=0.008).[6] Other studies evaluating outcomes of patients with m*BCOR* MDS are also limited by small sample size and inclusion of other gene mutations.[7,8]
In this study, we evaluated the genetic and clinical features, and factors predicting outcomes of patients with m*BCOR* AML/MDS.

Methods

Patients

The study was approved by the Mayo Clinic Institutional Review Board. Patients with WHO-defined myeloid neoplasms, including AML and MDS, and found to have a *BCOR* mutation on next-generation sequencing (NGS) performed between October 2015 to August 2021 on peripheral blood or bone marrow aspirate were included.[9] Patient demographics, disease characteristics at time of diagnosis and at the time of NGS testing, co-mutations, treatment-related variables and survival outcomes were extracted.

Because patients with m*BCOR* AML would be considered to have high-risk disease by ELN 2022 risk stratification, the disease risk for baseline characteristics was determined using the Revised International Prognostic Scoring System (IPSS-R) for MDS and the ELN 2017 risk stratification for AML.[3,10,11] However, the survival outcomes of patients with m*BCOR* AML was compared to a control cohort by both ELN 2017 and ELN 2022 risk stratification.

Consecutive patients who had NGS testing performed from May 2015 to September 2017, and had wild-type (wt) *BCOR* were considered as control cohort. Data on co-mutations, cytogenetics, and disease risk stratification by ELN 2017, ELN 2022 and IPSS-R criteria were collected for patients in the control cohort.

For patients undergoing alloSCT, a hematopoietic cell transplantation-comorbidity index (HCT-CI) score ≥3 was considered high. Conditioning regimen intensity was defined per the Center for International Blood and Marrow Transplant Research (CIBMTR) criteria.[12] Acute graft-*versus*-host disease (GvHD) was graded according to Glucksberg criteria and severity of chronic GvHD was determined using the 2014 National Institute of Health consensus criteria.[13,14] Relapse was defined as detection of disease after alloSCT by morphological, cytogenetic, or molecular analysis, as applicable. Relapse incidence (RI) was calculated from the time of alloSCT to the time of relapse detection.

Statistical analysis

Patients' and disease characteristics were summarized using descriptive statistics. The statistical comparison of categorical variables was performed using χ^2 test. For continuous variables, Kruskal-Wallis test was used for comparison of medians and *t* test was used for comparison of means.

Comparison of co-mutations among patients with wt*BCOR* *versus* m*BCOR* was performed using the "epitools" package and evaluated on log10 scale to prevent skewness.[15] Patients were deemed to enter the study at the time of NGS testing. Survival outcomes were analyzed in patients with AML/MDS from timepoint of study entry, unless mentioned otherwise. Kaplan-Meier and log-rank tests were used to estimate OS.[16] Median follow-up time was determined using the reverse Kaplan-Meier method.[17] Cox-proportional hazard model was used to determine the effect on OS. Allogeneic stem cell transplant (alloSCT) was considered a time-dependent covariate for both univariate and multivariate Cox proportional hazard analysis.[18]

The cumulative incidence of non-relapse mortality (NRM) and RI in patients undergoing alloSCT was determined using the competing risks method. Fine-Gray analysis was used to determine factors influencing NRM and RI post-alloSCT.

Only those genes that were mutated in at least 5 patients (approx. 5% of the entire cohort) were included in the univariate analyses. Because *BCOR* gene is located on X-chromosome, the variant allele frequency (VAF) of *BCOR* mutation was gender-adjusted for all the analyses. Variables with $P<0.20$ in univariate analyses were included in multivariate analysis. R 4.2.0 (R Foundation for Statistical Computing, Vienna, Austria) was used to perform all the statistical analyses. $P<0.05$ was considered statistically significant.[19,20]

Results

Out of 6,887 consecutive NGS tests performed, 138 (2%) patients were found to have *BCOR* mutation. These patients were compared to 275 patients with wt*BCOR*, who were considered to be the control cohort.

Control cohort

The control cohort consisted of 155 (82 males, 53%) patients with AML, 105 (71 males, 68%) with MDS, and 15 (5.5%) others. Median age at study entry was 74 years (interquartile range [IQR] 67-78 years) for MDS and 65 years (IQR 56-73 years) for AML.

Of the 155 AML patients, 17 (11%) were favorable risk, 61 (39%) were intermediate risk, and 73 (47.1%) were adverse risk by ELN 2022 criteria. By ELN 2017 criteria, 20 (13%) patients had favorable risk, 66 (42%) had intermediate risk, and 65 (42%) patients had adverse risk disease (Table 1). Risk stratification could not be determined for 4 (2.6%) patients. The most commonly mutated gene among patients with AML was *DNMT3A* (N=33, 21.3%), followed by mutations in genes *TET2* (N=29, 18.7%), *TP53* (N=27, 17.4%), and *IDH2* (N=27, 17.4%) (*Online Supplementary Table S1*). Among the 105 MDS patients, 27 (25%) were high or very high-risk by the IPSS-R. Fourteen (13%) patients had MDS with increased blasts-type 1 (MDS-IB1), while 15 (14%) patients had MDS-IB2. The most commonly mutated gene among MDS patients was *ASXL1* (N=29, 27.6%), followed by mutations in genes *TET2* (N=21, 20%) and *SF3B1* (N=20, 19%). *TP53* gene was mutated in 15 (14.3%) patients (*Online Supplementary Table S1*).

Table 1. Baseline characteristics of patients stratified by wild-type *BCOR* and mutated *BCOR*.

	wt*BCOR*, N=260	m*BCOR*, N=99	*P*
Median age in years (Min, Max)	69.4 (15.5, 88.4)	71.5 (33.8, 93.1)	0.01
Gender, N (%)			
Female	107 (41.2)	29 (29.3)	0.051
Male	153 (58.8)	70 (70.7)	
Diagnosis, N (%)			
AML	155 (59.6)	36 (36.4)	<0.001
MDS	105 (40.4)	63 (63.6)	
ELN 2017 risk stratification, N (%)			
Favorable	20 (12.9)	1 (2.8)	0.16
Intermediate	66 (42.6)	14 (38.9)	
Adverse	65 (41.9)	19 (52.8)	
Missing	4 (2.6)	2 (5.6)	
IPSS-R risk stratification, N (%)			
Very low	10 (9.5)	11 (17.5)	0.004
Low	29 (27.6)	7 (11.1)	
Intermediate	16 (15.2)	13 (20.6)	
High	11 (10.5)	20 (31.7)	
Very high	16 (15.2)	10 (15.9)	
Missing	23 (21.9)	2 (3.2)	
MDS (by blasts), N (%)			
IB1	14 (13.3)	20 (31.7)	<0.001
IB2	15 (14.3)	23 (36.5)	
MDS	72 (68.6)	17 (27.0)	
Missing	4 (3.8)	3 (4.8)	
Genes mutated, N (%)			
ASXL1	54 (20.8)	24 (24.2)	0.57
CALR	1 (0.4)	0 (0)	1
CBL	8 (3.1)	1 (1.0)	0.46
CEBPA	9 (3.5)	4 (4.0)	1
CSF3R	2 (0.8)	0 (0)	0.93
DNMT3A	41 (15.8)	28 (28.3)	0.01
ETV6	2 (0.8)	0 (0)	0.93
EZH2	10 (3.8)	5 (5.1)	0.83
FLT3	25 (9.6)	9 (9.1)	1
GATA2	7 (2.7)	4 (4.0)	0.75
IDH1	7 (2.7)	6 (6.1)	0.23
IDH2	29 (11.2)	13 (13.1)	0.74
JAK2	11 (4.2)	3 (3.0)	0.83
KIT	5 (1.9)	1 (1.0)	0.89
KRAS	6 (2.3)	5 (5.1)	0.31
MPL	2 (0.8)	0 (0)	0.93
NOTCH1	1 (0.4)	0 (0)	1
NPM1	21 (8.1)	0 (0)	0.008
NRAS	14 (5.4)	10 (10.1)	0.17
PHF6	8 (3.1)	5 (5.1)	0.56
PTPN11	8 (3.1)	3 (3.0)	1
RUNX1	34 (13.1)	45 (45.5)	<0.001
SETBP1	8 (3.1)	1 (1.0)	0.46
SF3B1	26 (10.0)	8 (8.1)	0.72
SRSF2	42 (16.2)	17 (17.2)	0.94
TET2	50 (19.2)	17 (17.2)	0.77
TP53	42 (16.2)	7 (7.1)	0.04
U2AF1	27 (10.4)	30 (30.3)	<0.001

wt: wild-type; m: mutated; N: number; AML: acute myeloid leukemia; MDS: myelodysplastic syndromes; ELN: European LeukemiaNet; IPSS-R: Revised International Prognostic Scoring System; IB: increased blasts.

Characteristics of patients with m*BCOR* acute myeloid leukemia and myelodysplastic syndromes

A total of 138 patients (96 males, 69.6%) with m*BCOR* hematologic disorders were evaluated. Thirty-six (26.1%) patients had AML and 63 (45.6%) had MDS (Table 2 and *Online Supplementary Table S2*). Among patients with AML and MDS, 64 (64.6%) had NGS testing performed before the first intervention/treatment.

Most of the patients (N=133, 96.4%) harbored only a single *BCOR* mutation. A total of 144 *BCOR* variants were seen across 138 patients. *RUNX1* (50/138, 36%) and *U2AF1* (38/138, 28%) were among the most commonly co-mutated genes (Figure 1A). Median VAF for *BCOR* mutation was 34.5% (IQR 16-61%) (Figure 1B); the median gender-adjusted VAF was 24.25% (IQR 11-37%). Mutations in the *BCOR* gene were present throughout the exon sequence and a particular hotspot could not be identified (Figure 1C). Frameshift variants were most common (82, 57%), followed by nonsense (45, 32%) and splice-site (16, 11%) variants, while only one (0.7%) patient had a missense mutation (Figure 1C).

Sixty-six (47.8%) patients had a normal karyotype: 16 (44.4%) among patients with AML, and 27 (42.9%) among patients with MDS (Figure 1D). None of the m*BCOR* AML or MDS patients had any abnormalities in chromosome 17. Monosomy 7 was found in 3 (4.8%) patients, and deletion 20q was seen in 8 (12.7%) patients with m*BCOR* MDS but were not seen in any patient with m*BCOR* AML. Other cytogenetic abnormalities among patients with m*BCOR* AML/MDS included trisomy 8 in 16 (16.2%) patients (7 AML, 43.8%; 9 MDS, 56.2%), deletion 5q in 8 patients (3 AML, 37.5%; 5 MDS, 62.5%), and inversion 3 in 2 (2%) patients (1 patient each with AML and MDS). One (1.59%) patient with MDS had rearrangement involving the *KMT2A* gene locus (11q23). Eighteen (13%) patients had complex karyotype: 7 (19.4%) among the 36 patients with AML and 6 (9.5%) among the 63 patients with MDS (Table 2, Figure 1D). Among the AML patients, one (2.8%) had favorable risk, 14 (38.9%) had intermediate risk, and 19 (52.8%) had adverse risk disease by ELN 2017 criteria; risk stratification could not be evaluated in 2 (5.6%) patients. Among the MDS patients, 43 (68.3%) had an intermediate or higher risk disease by IPSS-R; 20 (31.7%) patients had MDS-IB1, while 23 (36.5%) had MDS-IB2 (Table 2). Median white blood cell (WBC) count was $2.5x10^6$ cells/L (range 0.9 – $98.4x10^6$) in patients with AML and $2.30x10^6$ cells/L (range 0.8 – $14.4x10^6$) in patients with MDS.

Among AML and MDS patients, *BCOR* mutations were found to be highly associated with mutations in *RUNX1* gene (odds ratio [OR] 5.5; *P*<0.001). *BCOR* mutations were less commonly associated with *TP53* mutations, particularly among patients with MDS (OR 0.21; *P*=0.02) (Figure 1E, *Online Supplementary Table S3*). Patients with m*BCOR* MDS were found to be significantly associated with *U2AF1* mutation (OR 3.95; *P*<0.001). The *U2AF1* S34F variant was the predominant mutation (20/24, 83%) in m*BCOR* MDS patients (*Online Supplementary Table S4*). Interestingly, none of the patients with m*BCOR* had an *NPM1* co-mutation.

Survival analysis

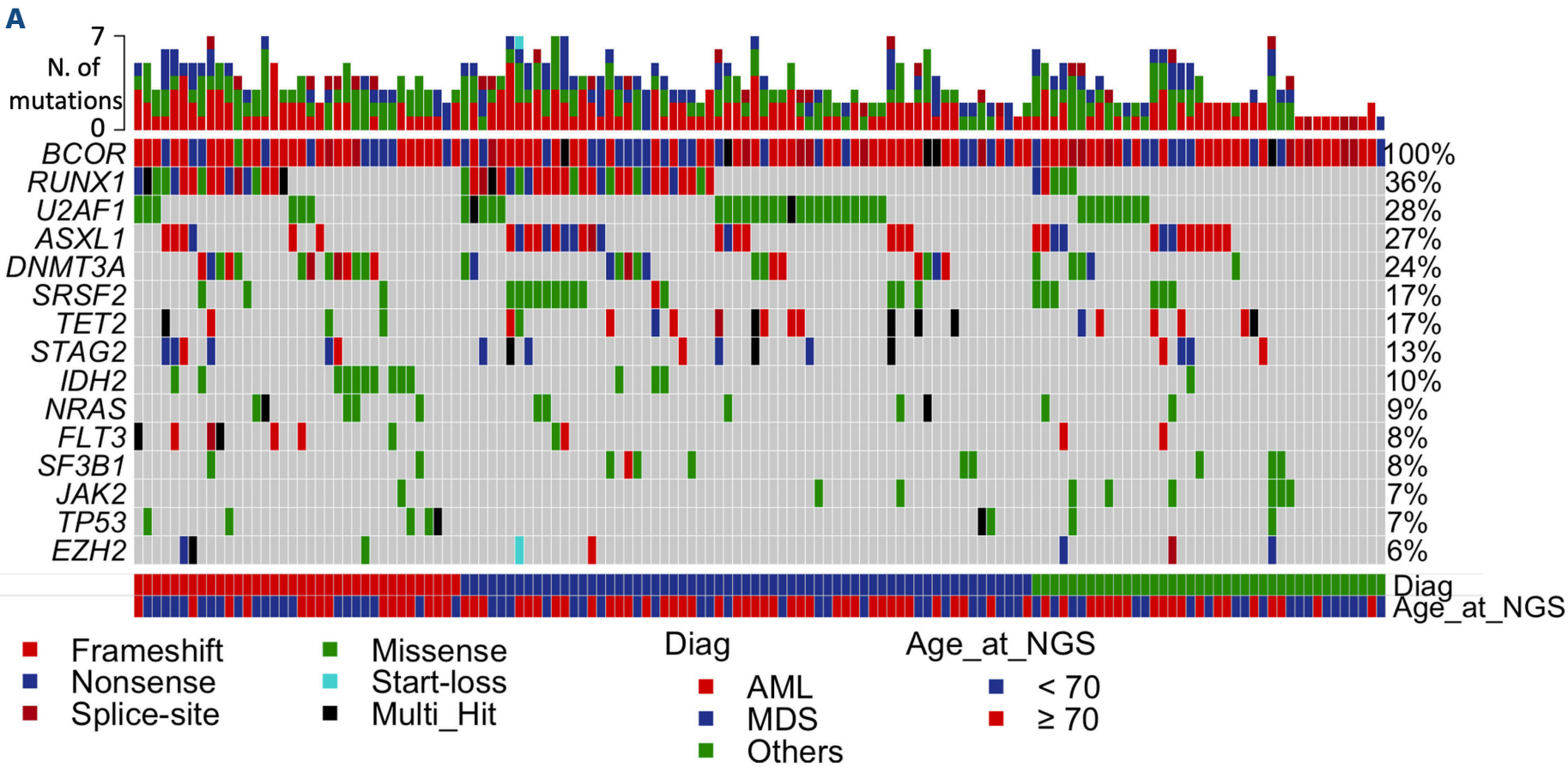
Median follow-up time after study entry among patients with m*BCOR* AML/MDS was 3.2 years (95% CI: 2.34-3.82 years). Median survival for the entire cohort of m*BCOR* AML/MDS patients was 15 months. The survival was lower among patients with AML compared to MDS, but this difference was not statistically significant (median OS: 0.74 *vs*. 1.53 years; *P*=0.21) (Figure 2A, *Online Supplementary Figure S1A*). Complex karyotype (median OS 0.3 *vs*. 1.5 years; *P*<0.001) and age ≥70 years (median OS 0.88 *vs*. 2.04 years; *P*=0.008) were associated with a worse survival (Figure 2B, C). Patients with m*BCOR* had similar survival regardless of the presence of additional other high-risk co-mutations by ELN 2022 criteria (median OS 1.26 *vs*. 1.3 years; *P*=0.46) (Figure 2D). The median OS from diagnosis among patients with NGS at diagnosis was similar to those who had NGS performed after treatment (median OS 2.07 *vs*. 2.05 years; *P*=0.87).

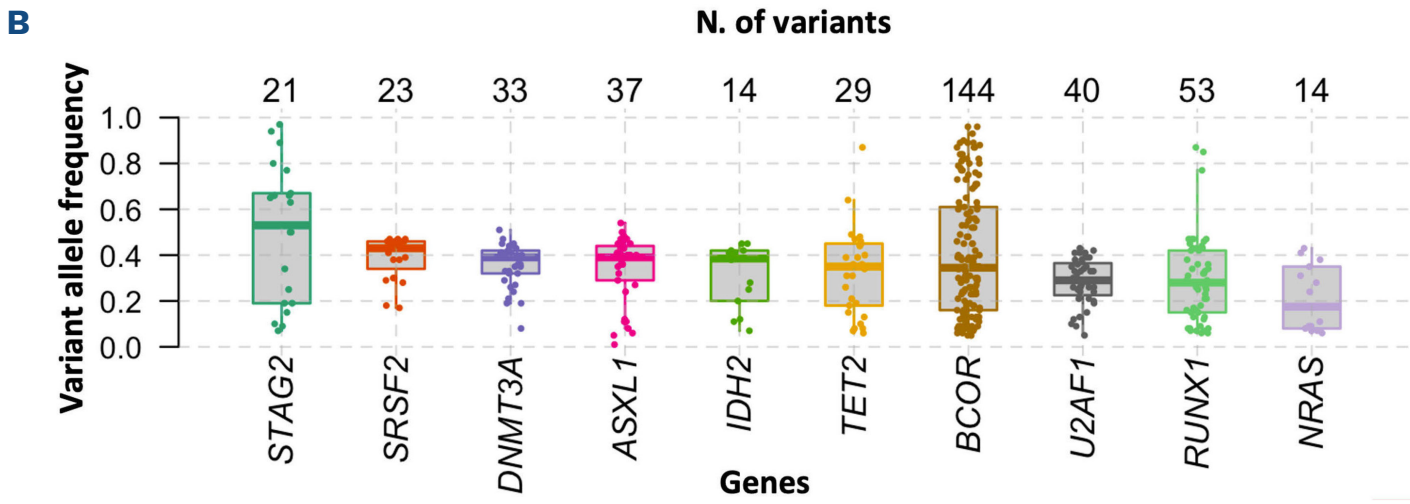
In comparison to wt*BCOR* patients (control cohort), survival since study entry among m*BCOR* patients was similar to those with adverse risk AML and numerically inferior to those with favorable or intermediate risk AML by the ELN 2017 criteria (3-year OS 29.5% *vs*. 24.4% *vs*. 41.2%; *P*=0.32) (Figure 2E). Survival from diagnosis among m*BCOR* AML patients was similar to those with adverse risk AML by ELN 2017 criteria and significantly inferior compared to those with favorable or intermediate risk AML by the ELN 2017 criteria (3-year OS 36.5% *vs*. 33.5% *vs*. 52.8%; *P*=0.03) (Figure 2F); similar findings were seen on evaluating survival by ELN 2022 criteria (*Online Supplementary Figure S1B, C*). Patients with m*BCOR* AML with NGS performed at diagnosis or after treatment had survival rates similar to adverse risk AML and inferior to favorable/intermediate risk AML by ELN 2017 criteria (3-year OS rate: 35.4% *vs*. 38.1% *vs*. 33.5% *vs*. 52.8% for m*BCOR* at diagnosis, m*BCOR* after treatment, wt*BCOR* with adverse risk AML and wt*BCOR* with favorable/intermediate risk AML, respectively; *P*=0.046). Survival was similar among m*BCOR MDS* with or without a *U2AF1* mutation (*P*=0.89) (*Online Supplementary Figure S2A*) and those with m*BCOR* AML/MDS with or without a *RUNX1* co-mutation (*P*=0.51) (*Online Supplementary Figure S2B*). Univariate analysis showed that, among patients with m*BCOR*, complex karyotype (HR 3.17, 95% CI: 1.69-5.96; *P*<0.001) and age ≥70 years at study entry (HR 2.04, 95% CI: 1.2-3.47; *P*=0.008) were associated with inferior survival, whereas alloSCT (HR 0.25, 95% CI: 0.11-0.57; *P*=0.001) was associated with an improved OS. Complex karyotype, age ≥70 years at study entry, alloSCT, and presence of EZH2 and/or KRAS co-mutations were factors included in the multivariate analysis (*Online Supplementary Table S5*). Multivariate analysis confirmed that complex karyotype was associated with a worse survival (HR 4.92, 95% CI:

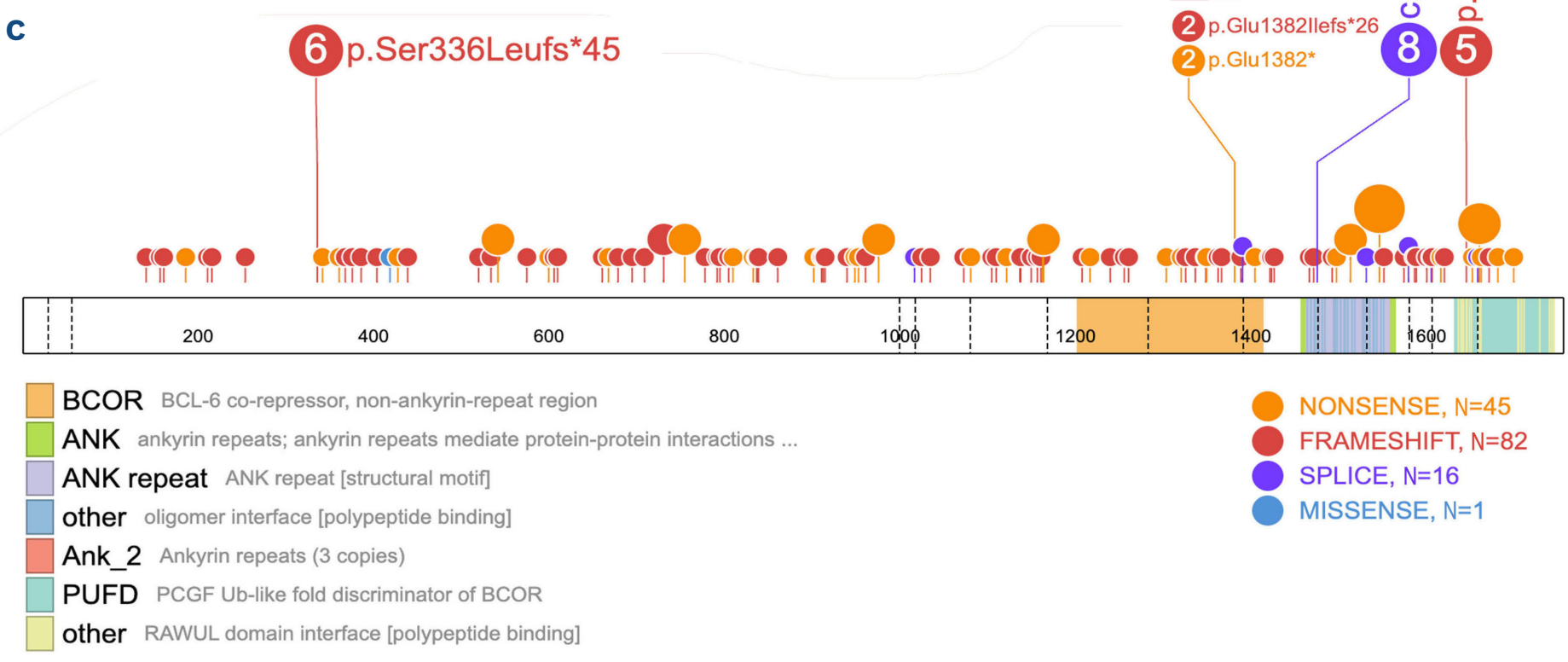
Table 2. Characteristics of patients with mutated *BCOR*.

	AML, N=36	MDS, N=63	Others, N=39
Age at diagnosisin yrs, median (IQR)	68 (63-80)	72 (65-77)	66 (56-76)
Gender, N (%)			
Female	12 (33.3)	17 (27.0)	13 (33.3)
Male	24 (66.7)	46 (73.0)	26 (66.7)
Ethnicity, N (%)			
Caucasian	34 (94.4)	58 (92.1)	32 (82.1)
Others	2 (5.6)	5 (7.9)	7 (17.9)
ELN 2017 risk stratification, N (%)			
Favorable	1 (2.8)	-	-
Intermediate	14 (38.9)	-	-
Adverse	19 (52.8)	-	-
Missing	2 (5.6)	-	-
IPSS-R risk stratification, N (%)			
Very low	-	11 (17.5)	-
Low	-	7 (11.1)	-
Intermediate	-	13 (20.6)	-
High	-	20 (31.7)	-
Very high	-	10 (15.9)	-
Missing	-	2 (3.2)	-
MDS by blasts, N (%)			
MDS-IB1	-	20 (31.7)	-
MDS-IB2	-	23 (36.5)	-
MDS	-	17 (27)	-
Missing	-	3 (4.8)	-
BM blasts %, median (IQR)	40 (26.5-65.5)	10.6 (5-13)	4 (0-7)
Hb≥10 g/dL , N (%)			
No	26 (72.2)	40 (63.5)	26 (66.7)
Yes	10 (27.8)	23 (36.5)	13 (33.3)
Platelets ≥ 100x10^9/L, N (%)			
No	21 (58.3)	44 (69.8)	24 (61.5)
Yes	15 (41.7)	19 (30.2)	15 (38.5)
Cytogenetics, N (%)			
Monosomy 7	0 (0)	3 (4.8)	1 (2.6)
Abnormal chromosome 7	4 (11.1)	7 (11.1)	2 (5.1)
Abnormal chromosome 17	0 (0)	0 (0)	1 (2.6)
Complex karyotype	7 (19.4)	6 (9.5)	5 (12.8)
Monosomal karyotype	1 (2.8)	3 (4.8)	3 (7.7)
Missing	2 (5.6)	3 (4.8)	2 (5.1)
BM blasts ≥ 10% at NGS, N (%)			
No	2 (5.6)	35 (55.6)	28 (71.8)
Yes	33 (91.7)	26 (41.3)	7 (17.9)
Missing	1 (2.8)	2 (3.2)	4 (10.3)
Multi-mutated *BCOR*, N (%)			
No	36 (100)	59 (93.7)	38 (97.4)
Yes	0 (0)	4 (6.3)	1 (2.6)
BCOR mutation type, N (%)			
Frameshift	23 (63.9)	36 (57.1)	21 (53.8)
Nonsense	10 (27.8)	22 (34.9)	11 (28.2)
Splice-site	2 (5.6)	6 (9.5)	8 (20.5)
Missense	1 (2.8)	0 (0)	0 (0)

AML: acute myeloid leukemia; MDS: myelodysplastic syndromes; N: number; yrs: years; IQR: interquartile range; ELN: European LeukemiaNet; IPSS-R: Revised International Prognostic Scoring System; IB: increased blasts; BM: bone marrow; Hb: hemoglobin; NGS: next-generation sequencing.







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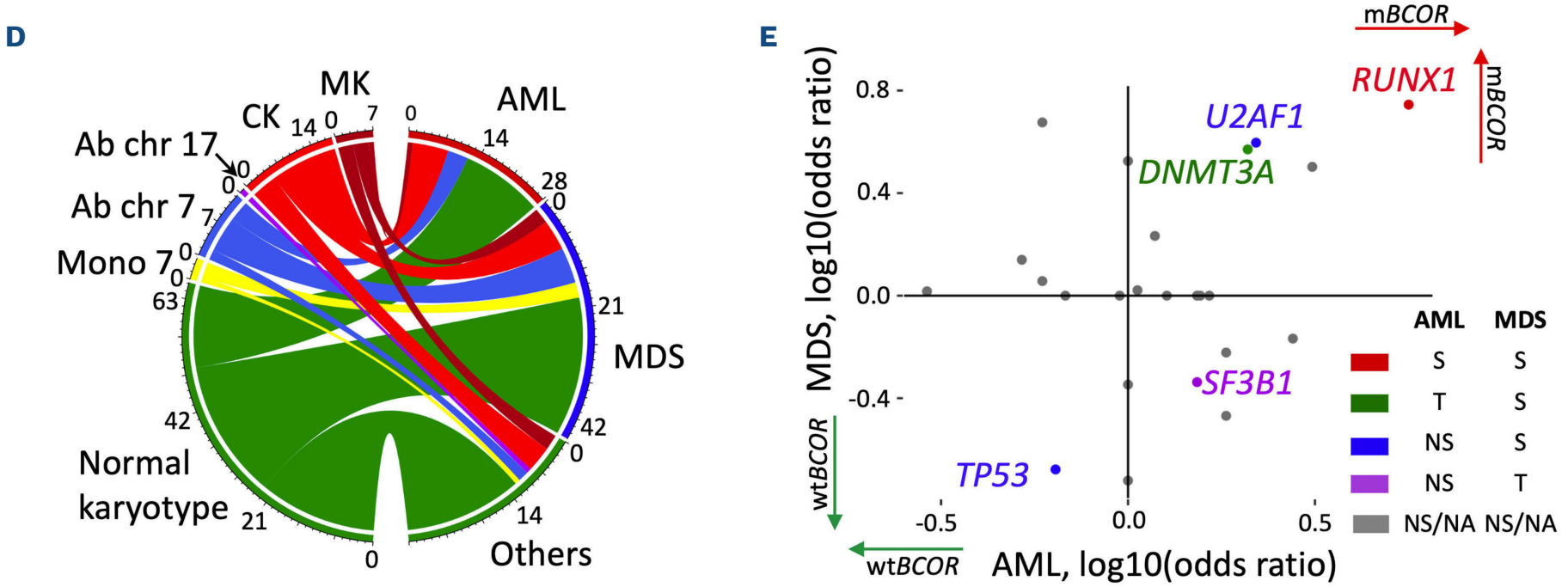

Figure 1. Genomic landscape of patients with *BCOR* mutations. (A) Oncoplot of patients with *BCOR* mutation; top 15 mutated genes are shown. (B) Top 10 genes with highest median variant allele frequency. (C) Lollipop plot showing various mutations across the *BCOR* gene. Mutations were most commonly frameshift or nonsense, and spread across the entire gene, without a specific hotspot region. (D) Specific cytogenetic abnormalities stratified by diagnoses: acute myeloid leukemia (AML), myelodysplastic syndromes (MDS), or others. Approximately half of the patients had normal karyotype, whereas complex karyotype or chromosome 17 abnormalities were seen in only a minority of patients. (E) Association of *BCOR* mutation with other genes among patients with MDS and AML. CK: complex karyotype; MK: monosomal karyotype; Ab: abnormal; Chr: chromosome; Diag: diagnosis; Mono: monosomy; N: number; NGS: next-generation sequencing; S: significant (P<0.05); T: trend (0.10 > P≥0.05; NS: not significant (P≥0.10); NA: not applicable.

2.0811.65; P<0.001), whereas alloSCT was associated with an improved survival (HR 0.38, 95% CI: 0.14-0.996; P=0.049) (*Online Supplementary Figure S1D*). Upon adjusting the *BCOR* VAF for gender, a trend towards inferior survival was seen among patients with *BCOR* VAF ≥40% (Figure 3).

Transplant outcomes

A total of 30 patients (19 males, 63.3%) underwent alloSCT after study entry. Median age at alloSCT was 65 years (IQR 58-68 years). Ten patients (33.3%) had AML, 17 (56.7%) had MDS, 2 (6.7%) had myeloproliferative neoplasm, and one patient (3.3%) had chronic myelomonocytic leukemia (CMML) (Table 3).

The 27 patients with m*BCOR* AML/MDS were evaluated for post-alloSCT outcomes. Median follow-up time after alloSCT was 2.5 years (95% CI: 1.8-4.0 years). Median HCT-CI score was 3 (IQR 1-3); thirteen (48.1%) patients had a high HCT-CI score. Fifteen (55.6%) patients were in complete remission (CR) at the time of alloSCT, 11 (40.7%) had persistent disease, and the disease status was not known for one patient (3.7%). Among the 10 patients with AML, 5 (50%) had pre-alloSCT minimal residual disease (MRD) testing performed, 4 (40%) of whom were MRD negative and one (10%) patient was MRD positive before transplant. Median survival after alloSCT was not reached; OS rate at three years was 61.1% (Figure 4A). A total of 8 (29.6%) deaths were reported within three years after transplant, of whom 5 patients (62.5%) had NRM and 3 (37.5%) patients died after relapse. The cumulative incidence of NRM was 23.7% and RI was 24.1% at three years after transplant (*Online Supplementary Figure S3*). Among the 5 patients with NRM, 2 (40%) died of infection, one (20%) died of GvHD, and the cause of death was not known for 2 (40%) patients. There was no significant difference in 3-year NRM (10% *vs*. 34.5%; P=0.32) and relapse (10% *vs*. 33.3%; P=0.24) in patients with AML or MDS (Figure 4B).

Given that *BCOR* is a high-risk mutation, we evaluated factors influencing post-transplant relapse. Univariate competing risk analysis showed that mutation in genes *ASXL1*, *RUNX1* (*mRUNX1*), melphalan-, or busulphan-based conditioning, and reduced-intensity conditioning were factors significantly affecting post-transplant relapse (*Online Supplementary Table S6*). Multivariate analysis showed that m*RUNX1* was associated with an increased risk of post-transplant relapse (HR 88.0, 95% CI: 1.98-3918; P=0.02), and melphalan-based conditioning was associated with a decreased risk of post-transplant relapse (HR 0.02, 95% CI: 0.001-0.40; P=0.01) (Figure 4C).

Patients with m*BCOR* AML undergoing alloSCT had a superior post-alloSCT survival compared to those with m*BCOR* MDS, but it was not statistically different (3-year OS 88.9% *vs*. 43.2%; P=0.1) (Figure 5A), while complex karyotype was associated with an inferior post-alloSCT survival (median OS 1.24 *vs*. NA years; P=0.04) (Figure 5B). Multivariate analysis that included complex karyotype and diagnosis (AML *vs*. MDS) (*Online Supplementary Table S7*) did not

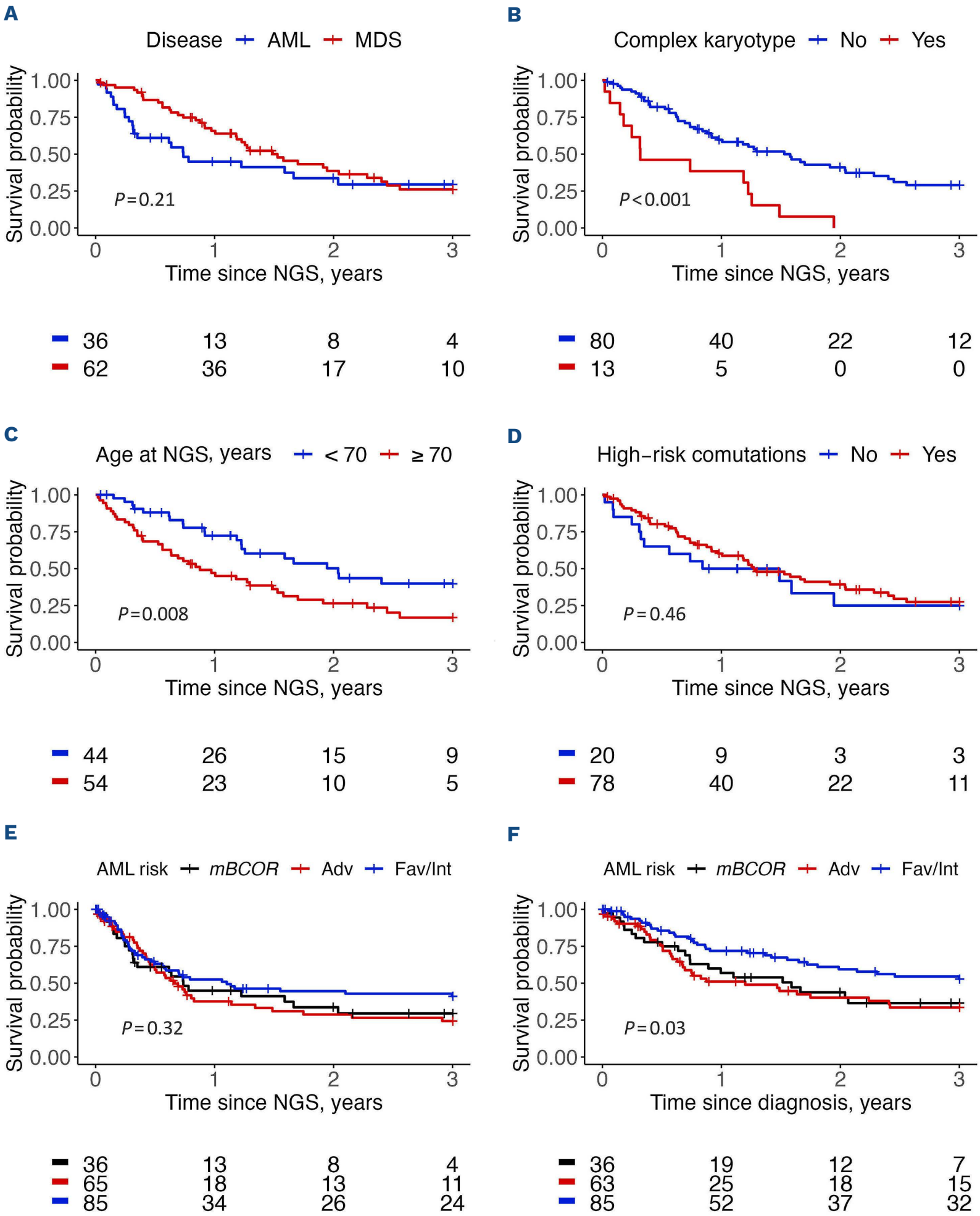

Figure 2. Clinical outcomes of patients with *BCOR*-mutated acute myeloid leukemia or myelodysplastic syndromes. Kaplan-Meier plots for survival since time of *BCOR* mutation (m*BCOR*) detection stratified by (A) disease type, (B) complex karyotype at next-generation sequencing (NGS), (C) age at NGS, and (D) presence/absence of high-risk co-mutations by European LeukemiaNet (ELN) 2022 criteria. (E and F) Overall survival of patients with mutated (m)*BCOR versus* wild-type (wt)*BCOR*, stratified by ELN 2017 risk category. Adv: adverse risk; AML: acute myeloid leukemia; Fav/Int: favorable / intermediate; MDS: myelodysplastic syndromes.

Variable	HR	95%CI	P
alloSCT	0.38	0.14-0.996	0.049
Complex karyotype	4.93	2.08-11.65	<0.001
ASXL1	1.3	0.67-2.49	0.436
TP53	0.97	0.32-2.95	0.961
EZH2	1.41	0.44-4.46	0.560
KRAS	1.6	0.42-6.09	0.487
Age ≥ 70 years at NGS	1.68	0.87-3.26	0.126
VAF (gender adj.) ≥ 40%	2.08	0.96-4.51	0.063
Splice-site mutation	0.41	0.12-1.36	0.146

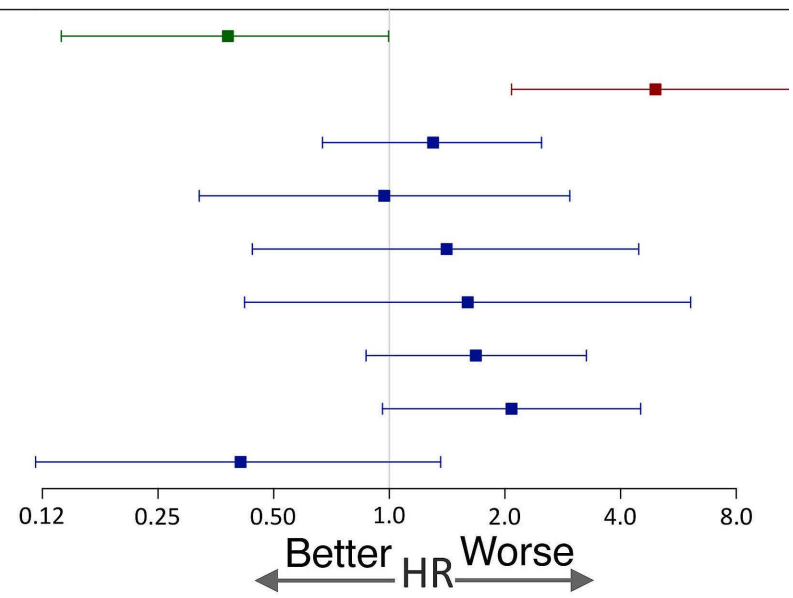

Figure 3. Multivariate analysis of factors affecting post-next-generation sequencing survival in patients with mutated *BCOR*. adj: adjusted; HR: Hazard Ratio; NGS: next-generation sequencing; VAF: variant allele frequency.

identify either of the 2 to be independently associated with post-alloSCT survival (Figure 5C), suggesting that alloSCT was beneficial regardless of phenotype or cytogenetics.

Characteristics of m*BCOR* myelodysplastic syndromes / myeloproliferative neoplasms overlap syndrome

A total of 11 (7.8%) patients had myelodysplastic syndrome/myeloproliferative neoplasm (MDS/MPN) overlap syndrome, 6 (54.5%) of whom had CMML. The median age at diagnosis was 75 years (IQR 68-82 years). Of the 11 patients, 8 (72.7%) patients had normal karyotype while trisomy 8 was seen in 2 patients (18.2%); karyotype was not available for one patient (9.1%). *ASXL1* (10 patients, 90.9%), *SRSF2* (5 patients, 45.5.%) and *RUNX1* (4 patients, 36.4%) were among the most commonly mutated genes. Two (18.2%) patients (1 CMML, 9.1%) harbored a mutation in the *TET2* gene. Median follow-up time was 1.6 years (95% CI: 1.57-NA). Survival was higher among patients with CMML compared to other MDS/MPN disorders; however, it was not statistically significant (median OS 15 *vs*. 9 months; *P*=0.5). Survival from NGS among patients with m*BCOR* MDS/MPN was similar to patients with mBCOR AML (median 7.5 *vs*. 9 months; *P*=0.28), but inferior to patients with m*BCOR* MDS (median 7.5 *vs*. 18.6 months; *P*=0.005) (*Online Supplementary Figure S4*).

Discussion

We described a cohort of 138 patients with m*BCOR*, of whom 99 (72%) had AML/MDS. Patients had *BCOR* mutations detected primarily in their 6th-7th decade of life and had low white blood cell (WBC) counts at diagnosis. These findings are parallel to those reported by Papaemmanuil *et al*.[4]

Studies have shown that a majority of patients with *BCOR* mutation have normal karyotype.[6,21] Approximately half of the patients in our cohort had normal karyotype and would have been classified in non-high-risk categories in the absence of m*BCOR*; complex karyotype was seen in only a minority of patients, while abnormality of chromosome 17 was not seen in any patient with m*BCOR* AML/MDS.

We could not identify a specific hotspot of mutations and the *BCOR* variants were spread throughout the gene. Most of the *BCOR* mutations were truncating mutations, and missense mutation was a rare finding in our cohort. This is similar to the findings of Grossmann *et al*., who evaluated 26 patients with m*BCOR* AML and found 15 frameshift, 6 nonsense, and 4 splice-site mutations, and a mutation pattern that is consistent with a tumor-suppressor function.[2,22,23]

Mutations in the *BCOR* gene have previously been associated with a *RUNX1* mutation.[6,21] Damm *et al*. also showed a strong association of m*BCOR* MDS with mutations in the *DNMT3A* gene.[6] Our study confirms these findings with a significant association of mutations in *BCOR* and *DNMT3A* in patients with MDS. A similar trend was also noticed in m*BCOR* AML in our cohort. Our study confirms that *BCOR* and *NPM1* mutations are mutually exclusive.[6,24]

We also found that m*BCOR* MDS were highly associated with mutations in the *U2AF1* gene. This is in contrast to the study by Montgomerry *et al*., which showed a predominant association of m*BCOR* with *mU2AF1* in lymphoid malignancies and not in myeloid neoplasms.[25] In our study, the *U2AF1* S34F was the predominant variant. The *U2AF1* has been shown to portend poor outcomes in patients with MDS.[26,27] Our study showed that the survival among m*BCOR* patients was poor, regardless of the presence or absence of the *U2AF1* mutation. More importantly, the poor survival among patients with m*BCOR* AML/MDS was

Table 3. Characteristics and outcomes of patients with m*BCOR* undergoing allogeneic stem cell transplantation.

	AML N=10	MDS N=17	Others N=3	*P*
Age at alloSCT in years				
Median (range)	65.6 (34.1-71.9)	65.6 (35.3-73.6)	66.4 (53.7-69.5)	
Gender, N (%)				
Female	4 (40.0)	6 (35.3)	1 (33.3)	0.96
Male	6 (60.0)	11 (64.7)	2 (66.7)	
HCT-CI score ≥3, N (%)	7 (70)	6 (35.3)	0 (0)	0.08
Missing	0 (0)	2 (11.8)	0 (0)	
Diagnosis, N (%)				
AML	10 (100)	NA	NA	NA
MDS	NA	17 (100)	NA	
CMML	NA	NA	1 (33.3)	
MPN	NA	NA	2 (66.7)	
ELN 2017 risk, N (%)				
Intermediate	3 (30.0)	NA	NA	NA
High	6 (60.0)	NA	NA	
Missing	1 (10.0)	NA	NA	
MDS IB-1 or IB-2, N (%)				
MDS	NA	3 (17.6)	NA	NA
MDS IB1	NA	7 (41.2)	NA	
MDS IB2	NA	7 (41.2)	NA	
High-risk co-mutations*, N (%)	7 (70.0)	11 (64.7)	3 (100)	0.47
CR at alloSCT	8 (80.0)	7 (41.2)	1 (33.3)	0.14
Missing	0 (0)	1 (5.9)	0 (0)	
Conditioning regimen, N (%)				
Busulfan-based	2 (20.0)	8 (47.0)	1 (33.3)	0.32
Melphalan-based	5 (50.0)	7 (41.2)	2 (66.7)	
TBI-based	3 (30.0)	1 (5.9)	0 (0)	
Missing	0 (0)	1 (5.9)	0 (0)	
Myeloablative conditioning, N (%)	2 (20.0)	5 (29.4)	1 (33.3)	0.8
Missing	0 (0)	1 (5.9)	0 (0)	
Major/bidirectional ABO mismatch, N (%)	1 (10.0)	1 (5.9)	0 (0)	0.83
Missing	0 (0%)	1 (5.9)	0 (0)	
CMV Donor neg./Recipient pos., N (%)	5 (50.0)	3 (17.6)	0 (0)	0.09
Missing	2 (20.0)	5 (29.4)	0 (0)	
GvHD prophylaxis, N (%)				
Methotrexate + CNI ± ATG	7 (70.0)	12 (70.6)	3 (100)	0.06
MMF + Tacrolimus	3 (30.0)	0 (0)	0 (0)	
PTCy	0 (0)	4 (23.5)	0 (0)	
Missing	0 (0)	1 (5.9)	0 (0)	
Acute GvHD, N (%)				
Grade 2-4	3 (30.0)	4 (23.5)	2 (66.7)	0.66
Grade 3-4	1 (10.0)	0 (0)	0 (0)	NA
Missing	2 (20.0)	7 (41.2)	0 (0)	
Chronic GvHD, N (%)				
Moderate-severe	3 (30.0)	2 (11.8)	0 (0)	0.36
Missing	0 (0)	1 (5.9)	0 (0)	

AML: acute myeloid leukemia; MDS: myelodysplastic syndromes; N: number; alloSCT: allogeneic stem cell transplantation; HCT-CI: hematopoietic cell transplantation-comorbidity index; NA: not available; CMML: chronic myelomonocytic leukemia; MPN: myeloproliferative neoplasms; ELN: European LeukemiaNet; CR: complete remission; IB: increased blasts; TBI: total body irradiation; CMV: cytomegalovirus; neg: negative; pos: positive; GvHD: graft-*versus*-host disease; CNI: calcineurin inhibitor; ATG: anti-thymocyte globulin; MMF: mycophenolate mofetil; PTCy: post-transplantation cyclophosphamide. *As per the European LeukemiaNet 2022 risk stratification.

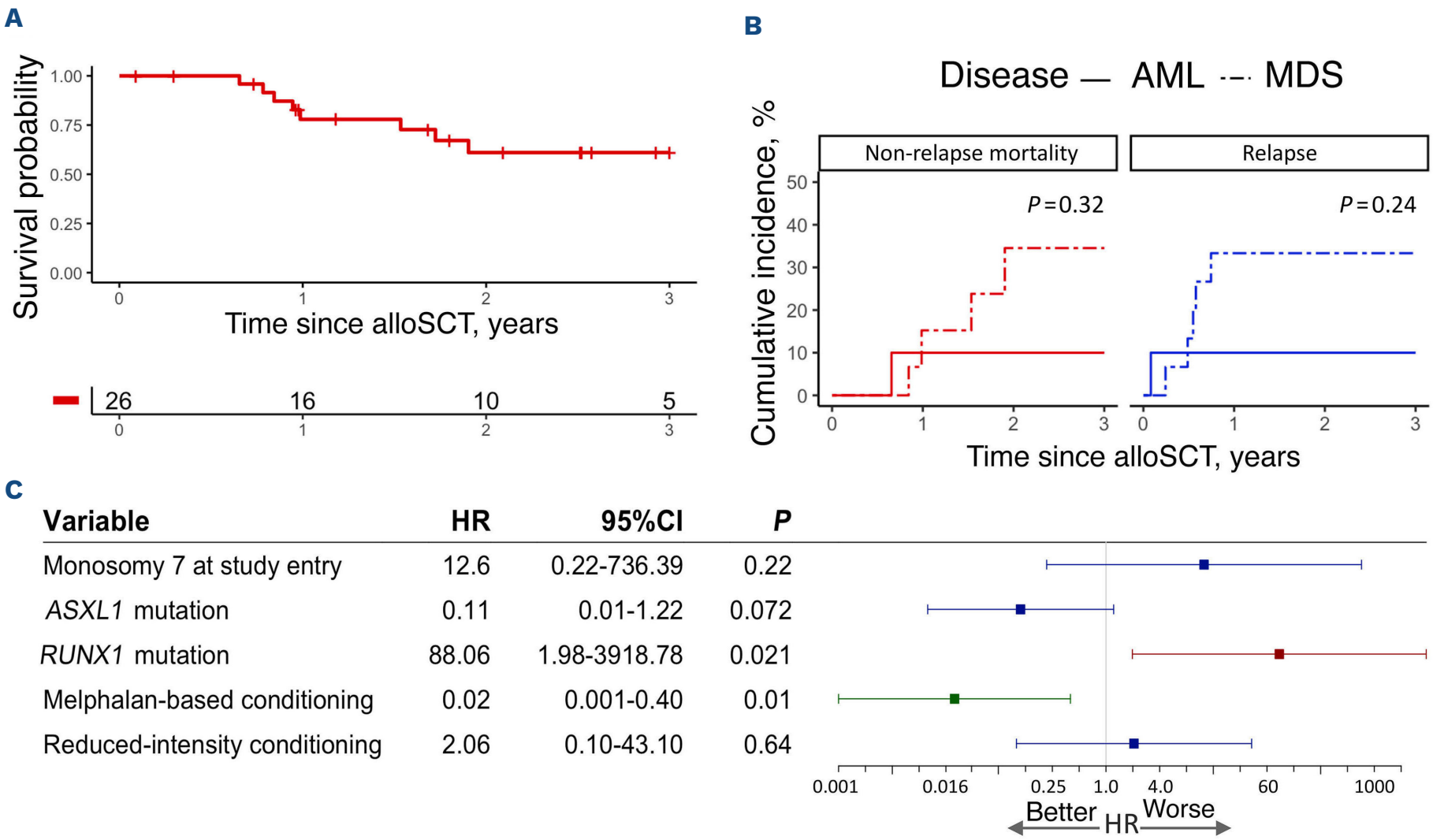

Figure 4. Outcomes of patients with *BCOR*-mutated acute myeloid leukemia / myelodysplastic syndromes undergoing allogeneic stem cell transplantation. (A) Overall survival after allogeneic stem cell transplantation (alloSCT). Follow-up data available in 26 patients. (B) Competing risk analysis showing cumulative incidence of non-relapse mortality (NRM) and relapse after alloSCT. (C). Multivariate competing risk analysis for relapse incidence at three years after alloSCT showing an overall adverse effect of *RUNX1* mutation and a positive effect of melphalan-based conditioning. AML: acute myeloid leukemia; HR: Hazard Ratio; MDS: myelodysplastic syndromes.

in spite of association with a high-risk co-mutation such as *RUNX1* or *ASXL1*.

Our study shows that patients with m*BCOR* AML/MDS have poor prognosis. The poor prognosis was seen across all the subgroups of MDS and AML patients, regardless of blast percentage. A comparison with the control cohort showed that survival among m*BCOR* AML patients parallels those with adverse risk AML patients by both ELN 2017 and ELN 2022 criteria, confirming the adverse prognosis of patients with m*BCOR* AML. Our study also shows that alloSCT improves survival in patients with m*BCOR* AML/MDS, and these patients should be evaluated for alloSCT at the earliest opportunity. More importantly, *RUNX1* co-mutation was associated with an increased risk of post-alloSCT relapse while melphalan-based conditioning was found to have a positive effect on post-alloSCT RI. A few studies have previously evaluated the effect of alloSCT in patients with m*BCOR* AML.[28,29] In a pooled cohort of *de novo* AML with secondary-type mutations including *BCOR*, Song *et al*. evaluated 15 patients undergoing alloSCT and found a cumulative RI of 33.3% at five years.[28] This parallels a 3-year RI of 24.1% reported in our study.

Some of the limitations of our study are that the NGS testing was done at different time points and the relatively small sample size of patients undergoing transplant. However, the former limitation is somewhat offset by the fact that the majority of patients with m*BCOR* AML/MDS in our cohort had NGS testing carried out before the first intervention/treatment. The time-to-event approach from NGS testing does lead to an underestimation of survival as the duration of disease before testing is not accounted for by this methodology. However, because this bias applies to both the m*BCOR* and the control cohort, its effect is diminished in a comparative analysis. For the same reason, we evaluated survival outcomes from both the time points, i.e., from diagnosis and from NGS. We further compared the survival outcomes of patients found to have a *BCOR* mutation at diagnosis *versus* later in the time-course and found similar survival rates. Given the rarity of *BCOR* mutations, a small sample size will be a limitation of any single-institutional study.

To our knowledge, this is the largest study specifically evaluating the impact of m*BCOR* across patients with AML and MDS. Our study also shows that the outcomes m*BCOR* AML/MDS are poor regardless of the presence or absence of high-risk co-mutations, suggesting an effect of *BCOR* in determining disease outcomes, while complex

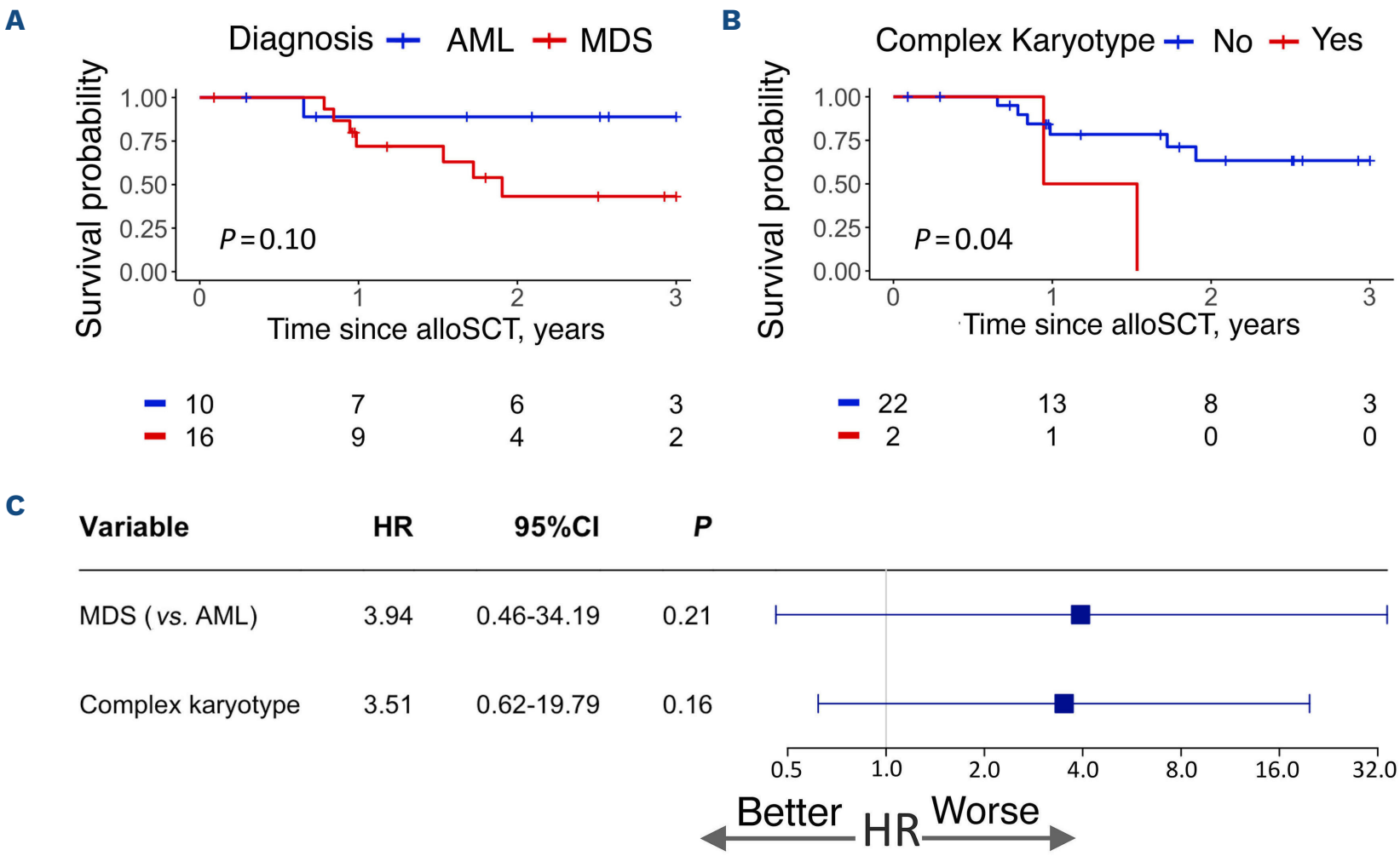

Figure 5. Factors influencing post-allogeneic stem cell transplantation survival in patients with mutated *BCOR* acute myeloid leukemia / myelodysplastic syndromes. Overall survival after allogeneic stem cell transplantation (alloSCT) stratified by: (A) disease type, (B) complex karyotype. (C) Multivariate analysis for 3-year post-alloSCT survival. AML: acute myeloid leukemia; HR: Hazard Ratio; MDS: myelodysplastic syndromes.

karyotype continues to have adverse outcomes. Finally, our study confirms that allogeneic transplant is associated with improved outcomes in patients with m*BCOR* AML/MDS; patients harboring a *RUNX1* co-mutation are at an increased risk of relapse, while melphalan-based conditioning decreases the relapse risk.

Disclosures

MVS reports research funding to the institution from Astellas, Abbvie, Celgene, and Marker Therapeutics. All of the other authors have no conflicts of interest to disclose.

Contributions

HBA, MVS and AB contributed to study design. AB, MG, RB and BK contributed to data acquisition and analysis. AB and HBA wrote the first draft. RH, DSV and PG performed molecular testing. JF, TB, HM, CAY, JP, AAM, MMP, MRL, WJH, KB, NG, AT, AA-K, MVS and HBA contributed patients and reviewed the manuscript. All authors approved the final version of the manuscript for publication.

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Figure 1C was created using ProteinPaint (https://proteinpaint.stjude.org/).

Data-sharing statement

Data may be obtained from the corresponding author upon reasonable request.

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