

Impact of splicing mutations in acute myeloid leukemia treated with hypomethylating agents combined with venetoclax

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Key Points

- Patients with spliceosome mutations have favorable overall outcomes with HMA+VEN therapy.
- Patients with mutated *U2AF1*, especially with concurrent *RAS* mutations, appear more resistant to HMA+VEN.

Spliceosome mutations (*SRSF2*, *SF3B1*, *U2AF1*, *ZRSR2*), are encountered in ~50% of secondary acute myeloid leukemia cases (sAML) and define a molecular subgroup with outcomes similar to sAML in de novo AML patients treated with intensive chemotherapy. Outcomes in patients with spliceosome mutations treated with hypomethylating agents in combination with venetoclax (HMA+VEN) remains unknown. The primary objective was to compare outcomes in patients with spliceosome mutations vs wild-type patients treated with HMA+VEN. Secondary objectives included analysis of the mutational landscape of the spliceosome cohort and assessing the impact of co-occurring mutations. We performed a retrospective cohort analysis of patients treated with HMA+VEN-based regimens at The University of Texas MD Anderson Cancer Center. A total of 119 patients (spliceosome mutated $n = 39$ [*SRSF2*, $n = 24$; *SF3B1*, $n = 8$; *U2AF1*, $n = 7$]; wild-type, $n = 80$) were included. Similar responses were observed between spliceosome and wild-type cohorts for composite complete response (CRc; 79% vs 75%, $P = .65$), and measurable residual disease–negative CRc (48% vs 60%, $P = .34$). Median overall survival for spliceosome vs wild-type patients was 35 vs 14 months ($P = .58$), and was not reached; 35 months and 8 months for patients with *SRSF2*, *SF3B1*, and *U2AF1* mutations, respectively. *IDH2* mutations were enriched in patients with *SRSF2* mutations and associated with favorable outcomes (1- and 2-year overall survival [OS] of 100% and 88%). *RAS* mutations were enriched in patients with *U2AF1* mutations and associated with inferior outcomes (median OS, 8 months). Comparable outcomes were observed between patients with vs without spliceosome mutations treated with HMA+VEN regimens, with specific co-mutation pairs demonstrating favorable outcomes.

Introduction

Mutations in genes encoding components of the spliceosome complex (*SRSF2*, *U2AF1*, *SF3B1*, *ZRSR2*) are identified in approximately one-third of patients with myelodysplastic syndrome (MDS) and nearly 50% of secondary acute myeloid leukemia cases evolving from MDS (sAML).^{1–4} Though less commonly encountered in de novo AML (~7%), splicing mutations (in particular *SRSF2*) correlate with inferior outcomes to standard induction therapy,^{1,5,6} mirroring a clinical course similar to

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patients with sAML.⁵ Contrary to these historically poor outcomes observed in patients with spliceosome mutations, mutations in genes involved in the cohesin complex (*STAG1*, *STAG2*, *SMC3*, *SMC1A*, *RAD21*), often considered alongside spliceosome mutations, appear to have an equivocal prognostic impact.⁷ The treatment landscape for AML now incorporates small molecule and molecularly targeted therapies, in which the differential influence of co-occurring mutations has resulted in increasingly challenging decision making in the management of AML. Understanding outcomes within these molecular subgroups is needed.

The incorporation of venetoclax (VEN) into low-intensity regimens has rapidly emerged as the standard of care for older, unfit patients with AML.⁸ Molecularly defined subgroups of patients with improved responses to VEN-based therapy have been defined.^{8,9} Certain molecular subgroups (*NPM1*, *IDH1/2*, or *TP53* mutated) appear predictive of VEN sensitivity or resistance, yet outcomes among other subgroups have not been described in depth. Although spliceosome mutations traditionally associate with inferior outcomes, VEN-based therapy may abrogate the negative prognostic impact of this molecular subgroup.¹⁰ In a correlative analysis of molecular determinates of outcomes in patients treated with hypomethylating agents (HMA) combined with VEN, gene mutations in *SRSF2* were the most commonly detected, and associated with a complete response (CR) rate of 65%.⁹ Additionally, patients with *SRSF2* mutations treated in the phase 1b Chemotherapy and Venetoclax in Elderly Acute Myeloid Leukemia (CAVEAT) trial investigating the addition of VEN to “5+2” anthracycline-based chemotherapy achieved higher blast reductions (median, 47%), translating into a favorable 82% overall response rate (ORR; CR + complete response with incomplete hematologic recovery [CRi]), and a median overall survival (OS) of 31 months.¹¹ Recent preclinical work similarly has suggested mutations in cohesin complex genes (in particular *RAD21*) result in increased VEN sensitivity in vitro.¹²

Understanding responses within specific subgroups of patients receiving VEN-based therapies is imperative for the identification of those patients most likely to benefit from novel combinations, in addition to those at increased risk of relapse. Although improved responses have been observed in prospective trials of VEN-based therapy, long-term outcomes in patients with spliceosome and/or cohesin mutations treated with VEN have yet to be fully reported. The primary aim of this study was to investigate outcomes in patients with spliceosome mutations treated with HMA+VEN at The University of Texas MD Anderson Cancer Center.

Methods

Study design and patient selection

Patients ≥ 18 years of age with a diagnosis of AML treated with frontline HMA+VEN-based therapy either on prospective clinical trial protocols (n = 98; NCT03404193, NCT02993523, NCT02203773) or off protocol (n = 21) at The University of Texas MD Anderson Cancer Center were included in this cohort analysis. Patients with acute promyelocytic leukemia were excluded. Patients with *FLT3*-ITD mutations receiving *FLT3* inhibitors in combination with HMA+VEN on protocol were included. Patients were stratified into spliceosome (presence of *SRSF2*, *SF3B1*, *U2AF1*, *ZRSR2* mutations) mutant and wild-type (ie, absence of spliceosome mutations) cohorts. Cohesin complex mutations (*STAG1*, *STAG2*,

SMC3, *SMC1A*, *RAD21*) were considered as a subgroup within the 2 larger cohorts for outcomes analyses. All included subjects were authorized after review of the MD Anderson Cancer Center institutional review board, and conducted under the Declaration of Helsinki.

Data collection and analysis

Patient data were reviewed for age at diagnosis, AML disease group (de novo, sAML, or therapy-related AML [tAML]), date of treatment initiation, therapy received, date of last follow-up, and survival status (living vs deceased) at last follow-up. Cytogenetics were performed using standard metaphase karyotype analysis in our Clinical Laboratory Improvement Amendments–certified laboratory. Molecular analysis was performed using next-generation sequencing (NGS) using a targeted 81-gene panel of recurrently mutated genes in myeloid malignancies as previously described.¹³ Measurable residual disease (MRD) was assessed using 8-color multiparameter flow cytometry (FC) using leukemia-associated immunophenotype or different from normal assessment with a minimum sensitivity of 10^{-3} to 10^{-4} (0.1%-0.01%). Patients were stratified by European LeukemiaNet (ELN) risk group according to 2017 ELN guidelines,¹⁴ and response criteria were defined per International Working Group criteria for AML¹⁵ (ORR: CR + CRi + complete response with partial hematologic recovery [CRh] + partial response + morphologic leukemia-free state; composite complete response [CRc]: CR + CRi + CRh). Time-to-event end points included OS (C1D1 until death), event-free-survival (EFS; C1D1 until relapse or death), and duration of response (DOR; time from achieving morphologic CRc until death or relapse).

Comparisons between cohorts for treatment response and clinical variables were determined using the χ^2 test or Fisher's exact test, whereas continuous variables were assessed using independent *t* test or the Wilcoxon rank-sum test as appropriate. Time-to-event analysis was assessed using the log-rank method, with logistical regression analysis and Cox proportional hazard modeling to determine the effect of variables on response as appropriate. False discovery rate (FDR)-adjusted *P* values using the Benjamini-Hochberg procedure were reported for survival analyses with multiple comparisons.

Results

Patient demographics are shown in Table 1. A total of 119 patients treated with HMA+VEN therapy were identified (azacitidine + VEN, n = 14; 10-day decitabine + VEN, n = 100; 5-day decitabine + VEN, n = 5). Thirty-nine patients (33%) had mutations in spliceosome genes (*SRSF2*, n = 24; *SF3B1*, n = 8; *U2AF1*, n = 7). Median age of the entire cohort was 72 years. Patients with spliceosome mutations were significantly older (median age 75 vs 70 years, *P* = .047), and more likely to be male (85% vs 40%, *P* < .01). The incidence of spliceosome mutations was 34% in de novo AML, 40% in sAML, and 24% in tAML. No significant differences were observed regarding patients diagnosed with sAML (21% vs 15%) or tAML (18% vs 28%) in the spliceosome vs wild-type cohort, respectively. The majority of patients in both cohorts had adverse risk disease by 2017 ELN criteria (spliceosome, 54%; wild-type, 64%).

Cytogenetic and molecular characteristics

The cytogenetic class of the cohorts is shown in Table 1. The majority of patients in the spliceosome cohort had intermediate-risk

Table 1. Patient demographics

Demographic	All patients (N = 119)	Spliceosome (n = 39)	Wild-type (n = 80)	P
Age, median (range), y	72 (40-89)	75 (56-85)	70 (40-89)	.047
Male, n (%)	65 (55)	33 (85)	32 (40)	<.01
ECOG, median (range)	1 (0-3)	1 (0-3)	1 (0-3)	.11
Median WBC (range), $\times 10^3/\mu\text{L}$	3.1 (0.3-81)	3.6 (0.3-81)	2.9 (0.5-43)	.61
Treatment duration, median (range), mo	5.7 (0.1-43)	5.5 (0.5-43)	5.7 (0.1-32)	.90
Treatment regimen, n (%)				
Azacitidine + venetoclax	14	3 (8)	11 (14)	.54
5-d decitabine + venetoclax	5	2 (5)	3 (4)	.66
10-d decitabine + venetoclax	100	34 (87)	66 (83)	.60
FLT3 inhibitor	14 (12)	2 (5)	12 (15)	.14
Disease, n (%)				
De novo AML	70 (59)	24 (62)	46 (58)	
sAML	20 (17)	8 (21)	12 (15)	.44
MDS	17	7	10	—
MPN	3	1	2	—
tAML	29 (24)	7 (18)	22 (28)	.36
ELN risk group, n (%)				
Favorable	28 (24)	11 (28)	17 (21)	.49
Intermediate	19 (16)	7 (18)	12 (15)	.79
Adverse	72 (61)	21 (54)	51 (64)	.32
Cytogenetic group, n (%)				
Diploid	39 (33)	14 (36)	25 (31)	.68
Other intermediate	30 (25)	14 (36)	16 (20)	.07
Adverse/complex	50	11 (28)	39 (49)	.047

ECOG, Eastern Cooperative Oncology Group; MPN, myeloproliferative neoplasm; WBC, white blood cell.

cytogenetics (72%; diploid, 36%; other intermediate, 36%), with a minority (28%) having adverse risk or complex cytogenetic findings. A larger proportion of wild-type patients had adverse risk cytogenetics or complex karyotype (49%, $P = .047$).

The molecular landscape of the spliceosome cohort and specific amino acid alterations associated with spliceosome mutations are shown in Figure 1A-B. *SRSF2* mutations predominantly resulted in well-described substitutions at proline residue 95 ($n = 23$, 96%),¹⁶ with only 1 patient demonstrating a p.A74G mutation (Figure 1B). *SF3B1* mutations occurred within the common K700 and K666 residues.¹⁶ Of interest, the majority (75%) were K666N, which have recently been associated with inferior outcomes in myeloid malignancies.¹⁷ Similar to *SRSF2*, all *U2AF1* mutations resulted in changes within the commonly identified S34 and Q157 residues within the first 2 zinc finger motifs of *U2AF1*.

Mutation differences between cohorts are shown in Figure 2A. Mutations in *DNMT3A*, *ASXL1*, *TET2*, *TP53*, *RUNX1*, *RAS*, and *NPM1* were prevalent at a frequency $\geq 15\%$ in both cohorts. *IDH* mutations (36% vs 15%) significantly associated with spliceosome mutations (Figure 2B), whereas *TP53*, *TET2*, and *EZH2* mutations were enriched in the wild-type cohort ($P < .05$).

Molecular differences between cohorts were also assessed by genetic class (ie, tumor suppressor, transcription factor, active

signaling, methylation, and chromatin modifying). Within the spliceosome cohort, enrichment was observed for mutations in methylation genes (64% vs 33%, $P < .01$). Mutations in chromatin modifiers (*ASXL1*, *BCOR*, *EZH2*; 40% vs 20%, $P = .04$) and tumor suppressor mutations (*TP53*, *WT1*, *PHF6*) were enriched within the wild-type cohort (45% vs 23%, $P = .03$); active signaling mutations (*FLT3*, *CBL*, *PTPN11*, *KINRAS*) demonstrated a trend toward enrichment in wild-type patients (55% vs 36%, $P = .05$). No significant difference was observed in transcription factor or cohesin complex mutations. Median mutation burden was not significantly different between cohorts (4 vs 4.5) or spliceosome mutations (*SRSF2*, 4; *SF3B1*, 3; *U2AF1*, 5; Figure 3A).

Mutational landscape within the spliceosome cohort

Co-mutation patterns in patients with *SRSF2* ($n = 24$), *SF3B1* ($n = 8$), and *U2AF1* ($n = 7$) mutations were further explored (Figure 2B). *SRSF2* mutations frequently co-occurred with mutations in *IDH2* (33%), *TET2* (29%), *NPM1* (29%), and *RUNX1* (25%). *IDH2* mutations were found nearly exclusively with *SRSF2* mutations compared with other spliceosome mutations (88% vs 11%) and were enriched compared with other mutational pairs (33% vs 9%, $P < .01$). *SF3B1* mutations co-occurred with mutations in *RUNX1* (50%), *NPM1* (38%), *DNMT3A* (25%), *TP53* (25%), and *IDH1* (25%). *U2AF1* mutations co-occurred with mutations in *RAS* (71%), *TET2* (57%), and *DNMT3A* (57%). Compared with other

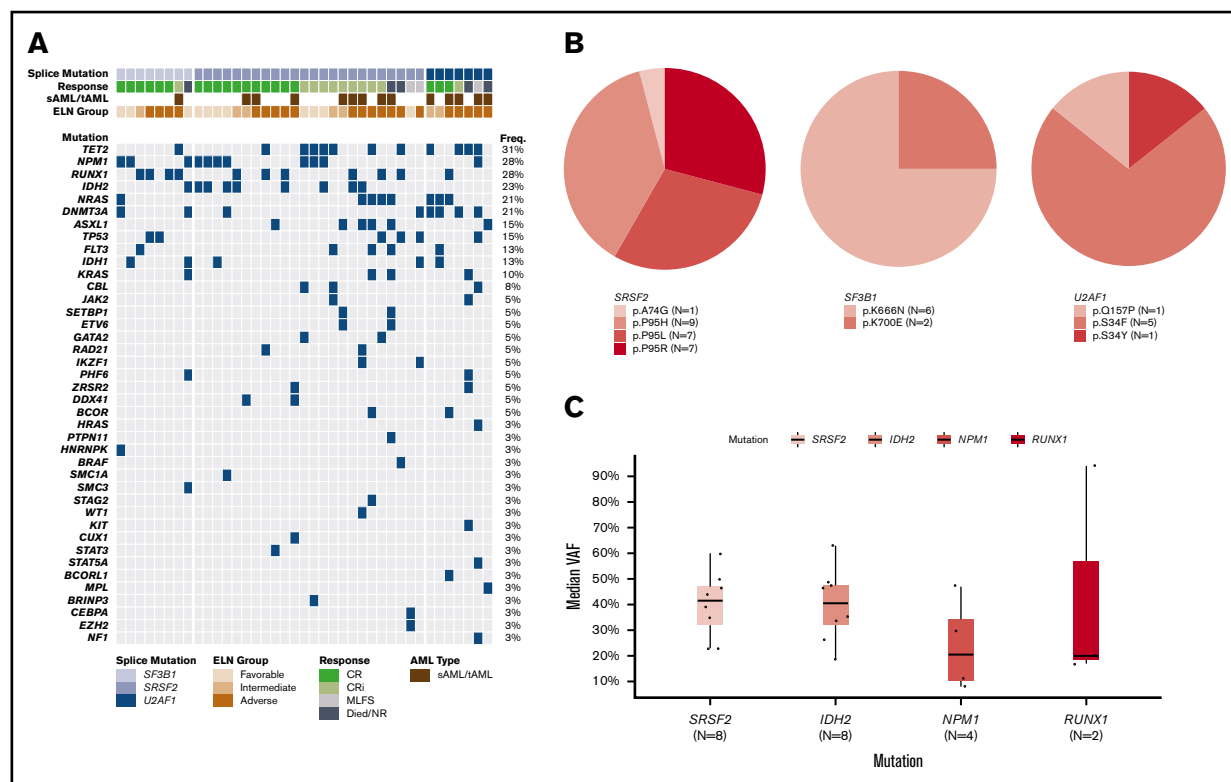


Figure 1. Molecular landscape of the spliceosome cohort. (A) OncoPrint of molecular mutations in patients with spliceosome mutations. (B) Frequency and associated amino acid substitution of included spliceosome mutations. (C) Variant allele frequency (VAF) of recurrently mutated genes in patients with *SRSF2* and *IDH2* co-mutations.

spliceosome patients, *RAS* mutations were enriched in patients with *U2AF1* mutations ($P < .01$).

Cohesin complex mutations have demonstrated preclinical sensitivity to VEN-based therapies¹² and are often considered in the context of spliceosome mutations. Cohesin complex mutations were identified in 14% ($n = 17$) of the study cohort. 13% ($n = 5$) of patients in the spliceosome cohort had co-occurring cohesin complex mutations. Among the 12 wild-type patients with cohesin complex mutations (*RAD21*, $n = 2$; *STAG1*, $n = 5$; *STAG2*, $n = 2$; *SMC1A*, $n = 2$; *SMC3*, $n = 1$), frequent co-occurring mutations included *TET2* (75%), *TP53* (50%), *NPM1* (33%), *RUNX1* (33%), *NOTCH1* (33%), *ASXL1* (25%), *DNMT3A* (25%), and *PTPN11* (25%).

Response to HMA+VEN

Response outcomes for the 2 cohorts are shown in Table 2 and Figure 3B. The ORR for the entire cohort was 81% (spliceosome, 89%; wild-type, 79%). CRc was achieved in 79% and 75% of patients in the spliceosome and wild-type cohorts. Spliceosome patients were more likely to achieve a CRi/CRh compared with wild-type patients (28% vs 11%, $P = .03$). Twenty-two patients (18%; spliceosome, $n = 5$ [13%] vs wild-type, $n = 17$ [21%]) were either refractory to therapy or deceased before disease assessment. Responses based on specific mutations are shown in Table 2. Patients with *NPM1* mutations were more likely (93% vs 71%, $P = .02$), and those with *TP53* mutations or adverse risk/complex cytogenetics less likely (*TP53*: 58% vs 84%, $P = .01$; adverse risk/complex cytogenetics: 64% vs 86%, $P = .01$) to achieve a CRc.

Despite the differential response observed to treatment of these specific mutations, spliceosome mutations had no significant influence on response within the respective cohorts. CRc rates for patients with *SRSF2*, *SF3B1*, and *U2AF1* mutations were 83%, 88%, and 57%.

Seventy-four (81%; spliceosome, $n = 24$; wild-type, $n = 50$) of the 91 patients achieving a CRc had adequate samples for MRD analysis via FC, 17 (19%) had limited/inadequate samples. MRD negative (MRD⁻) CR was attained in 54% ($n = 40$), including 42% ($n = 10$) of spliceosome and 60% ($n = 30$) of wild-type patients. Among evaluable patients in the spliceosome cohort, MRD⁻ CRc was observed in 56% ($n = 9/16$), 20% ($n = 1/5$), and 0% ($n = 0/3$) of those with *SRSF2*, *SF3B1*, and *U2AF1* mutations.

Patients with isolated cohesin complex ($n = 12$) or co-occurring cohesin complex and spliceosome mutations ($n = 5$), demonstrated a 71% CRc rate (cohesin complex, 67%; spliceosome/cohesin co-mutated, 80%). Eight patients with cohesin complex mutations had evaluable MRD samples, with 38% ($n = 3/8$) attaining an MRD⁻ CR.

Median variant allele frequency (VAF) at diagnosis in patients with *SRSF2*, *SF3B1*, and *U2AF1* mutations was 38%, 33%, and 43%, respectively (supplemental Figure 1). A minority (26%) of patients had NGS analysis performed in remission. Median VAF of *SRSF2*, *SF3B1*, and *U2AF1* mutations in remission were 17%, 6%, and 2%. In the 10 patients with spliceosome mutations who obtained an MRD⁻ CRc, paired NGS analysis performed in remission in 5 patients with *SRSF2* mutations demonstrated persistent *SRSF2*

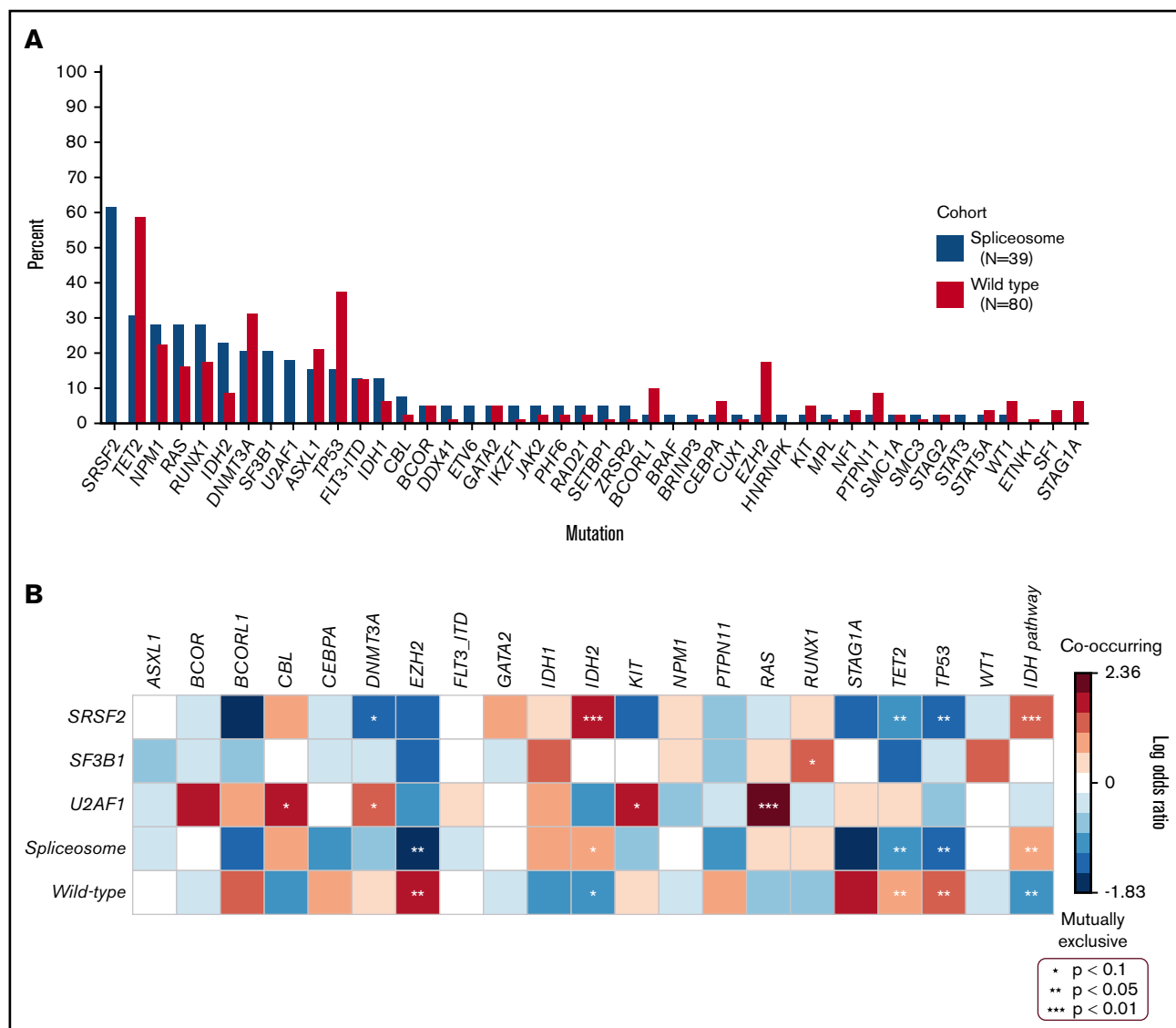


Figure 2. Co-mutation frequencies and enrichment in spliceosome-mutated and wild-type cohorts. (A) Mutation frequency in patients with vs without spliceosome mutations. (B) Heatmap demonstrating co-occurring mutations and mutually exclusive mutations between spliceosome mutations and cohorts, respectively.

mutations in 40% (n = 2), with a median remission *SRSF2* VAF of 23% in these 2 patients. Both patients with persistent *SRSF2* mutations demonstrated reductions in VAF on remission sampling. Notably, both patients (ages 71 and 72 years) had co-mutations in *IDH2*, which also persisted in remission. Patient 1 developed mutations in *ASXL1*, *TET2*, and *GNAS* in remission, consistent with emergence of novel clones. Patient 2 had a persistent *NPM1* mutation in remission, likely a molecular indicator of a residual leukemic clone, despite negative FC (supplemental Figure 2).

OS

Survival outcomes are shown in Table 3. After a median follow-up of 24 months, the median EFS and OS for the entire cohort was 10 (95% confidence interval [95% CI], 9-15) and 14 months (95% CI, 12-not reached [NR]). Median DOR was 11 (95% CI, 7-NR) and 15 (95% CI, 10-24) months for spliceosome, and wild-type patients, respectively. No significant difference in OS was observed between

patients with vs without spliceosome mutations (median OS, 35 [95% CI, 13-NR] vs 14 months [95% CI, 10-NR]; Figure 4A). One-year OS was 63% in the spliceosome cohort compared with 53% in the wild-type cohort. As shown in Figure 4B, median OS was NR (95% CI, 13-NR), 35 months (95% CI, 4-NR), and 8 months¹⁸ (95% CI, 3-NR) for patients with *SRSF2*, *SF3B1*, and *U2AF1* mutations, respectively. Despite the spliceosome cohort being significantly older, no significant difference in OS was observed in patients age ≥ 70 or < 70 years. Among responding patients with evaluable MRD (n = 74), achievement of MRD-negative CRc associated with improved DOR (median 19 vs 7 months, $P < .01$), EFS (median 20 vs 8 months, $P < .01$), and OS (median NR vs 12.6, $P = .035$; supplemental Figure 3). MRD-negativity demonstrated a trend toward improved OS in spliceosome patients (median, NR vs 14 months; $P = .08$), and resulted in numerically longer survival in wild-type patients (median, 20 vs 11 months; $P = .16$). The 2 patients described previously with persistent *SRSF2*

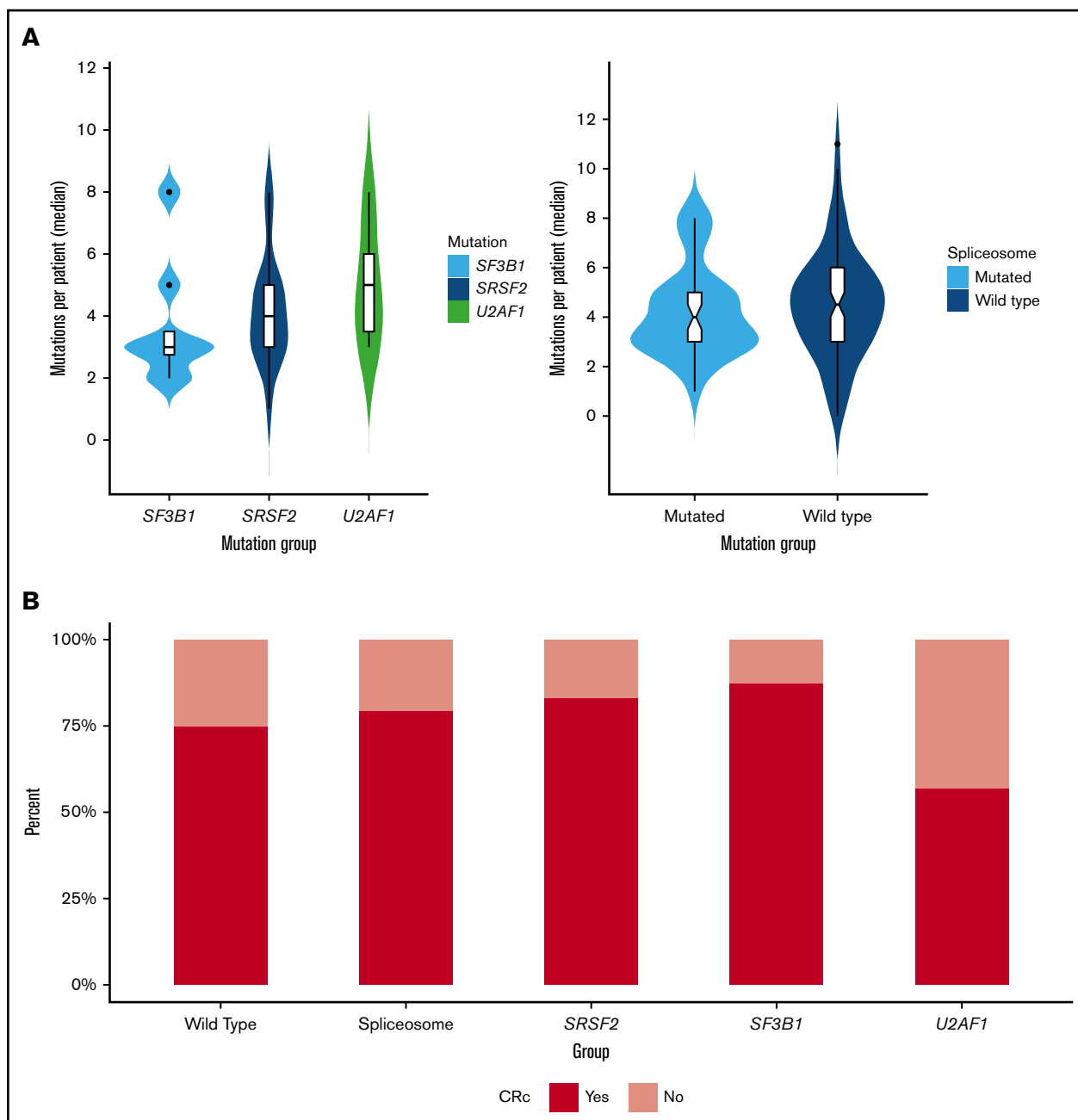


Figure 3. Mutation burden and treatment response in spliceosome-mutated and wild-type cohorts. (A) Median mutation burden in patients with *SF3B1*, *SRSF2*, and *U2AF1* mutations and in patients with vs without spliceosome mutations. (B) Composite CR rates in wild-type and spliceosome cohorts, and by individual spliceosome mutation.

mutations identified on NGS despite flow MRD⁻ CRc remain alive and in remission, after a median follow-up of 28 months.

Median OS had not been reached in patients with ELN favorable or intermediate-risk disease in either cohort. Wild-type patients with adverse risk disease had significantly inferior survival with a median OS of 10 months (95% CI, 8-18; $P < .01$). In both cohorts, only adverse cytogenetics associated with an inferior outcome (median OS spliceosome: 5 months, $P < .01$; median OS wild-type: 9 months, $P < .01$) because spliceosome and

wild-type patients with diploid or other intermediate cytogenetics had favorable OS (median OS spliceosome, diploid: NR vs other intermediate, 35 months; median OS wild-type, diploid: NR vs other intermediate, 19 months). Inferior OS was observed in patients with sAML or tAML compared with those with de novo disease in both cohorts (Table 3). No differential impact on survival was observed based on the presence or absence of spliceosome mutations in patients with sAML/tAML or within respective ELN or cytogenetic risk groups (supplemental Figure 4).

Table 2. Patient outcomes

Response	All patients (N = 119)	Spliceosome (n = 39)	No spliceosome (n = 80)	P
ORR	97 (81)	34 (89)	63 (79)	.32
CRc	91 (76)	31 (79)	60 (75)	.65
CR	71 (60)	20 (51)	51 (64)	.23
CRi/CRh	20 (17)	11 (28)	9 (11)	.03
MLFS	6 (5)	3 (10)	3 (4)	.39
NR/died	22 (18)	5 (13)	17 (21)	.32
Composite CR by molecular group				P*
<i>NPM1</i> (n = 29)	27 (93)	9/11 (82)	18/18 (100)	.02
<i>IDH1</i> (n = 10)	6 (60)	3/5 (60)	3/5 (60)	.24
<i>IDH2</i> (n = 16)	14 (88)	8/9 (89)	6/7 (86)	.35
<i>FLT3-ITD/TKD</i> (n = 20)	16 (80)	4/5 (80)	12/15 (80)	.78
<i>TP53</i> (n = 36)	21 (58)	3/6 (50)	18/30 (60)	.01
Adverse/complex cytogenetics (n = 50)	32 (64)	5/11 (45)	27/39 (69)	.01

Data are n (%) or n/N (%).

MLFS, morphologic leukemia-free state.

*P value for all patients (spliceosome and wild-type) within a classified molecular group.

Molecular determinates of outcomes

Outcomes were assessed further by mutational class. *NPM1* mutations (n = 29) were associated with a significant improvement in OS in the entire population (median OS, NR vs 12 months; $P < .01$). However, this survival difference was not significant in patients with spliceosome mutations (median OS, NR vs 14 months; $P = .48$) compared with wild-type (median OS, NR vs 10.4 months; $P < .01$) likely secondary to the low number of included patients. Methylation mutations (*DNMT3A*, *TET2*, *IDH1*, *IDH2*) demonstrated a trend toward improved OS in spliceosome patients (median OS, 35 vs 8 months; $P = .09$). Within the methylation group, this survival impact appeared largely driven by the presence of *IDH* mutations because no difference in OS was seen between those with or without methylation mutations when censoring patients with *IDH* mutations (median OS, 11 vs 8 months; $P = .84$). Tumor suppressor mutations (*TP53*, *WT1*, *PHF6*; n = 45) strongly associated with adverse outcomes in both spliceosome (median OS, 4 vs 35 months; $P < .01$) and wild-type patients (median OS, 8.5 vs NR; $P < .01$). No difference in OS was observed between patients with chromatin, active signaling, or transcription factor mutations irrespective of cohort.

Cohesin complex and spliceosome mutations

Patients with spliceosome mutations and/or cohesin complex mutations (n = 51) demonstrated a median OS of 35 months. When divided into by those with co-occurring cohesin and spliceosome mutations (n = 5), isolated spliceosome (n = 34), or isolated cohesin mutations (n = 12), no significant difference in OS was observed. Patients with co-occurring cohesin complex and spliceosome mutations had favorable 1-year survival of 80%, whereas those with isolated spliceosome or cohesin mutations had 1-year OS similar to that observed in wild-type patients (spliceosome, 58%; cohesin, 55%; wild-type, 53%; supplemental Figure 5).

Impact of co-mutations on outcomes to HMA+VEN

Patients with co-occurring spliceosome and *IDH1/2* mutations (n = 13), including 7 patients with co-occurring *NPM1* mutations,

demonstrated particularly favorable OS compared with patients with spliceosome mutations in the absence of *IDH1/2* (median OS NR vs 8 months, FDR-adjusted $P = .024$), with observed outcomes comparable to wild-type patients with *IDH1/2* mutations without spliceosome mutations (median OS, NR vs 22 months; FDR-adjusted $P = .18$) (Figure 4C). Similar to wild-type patients with *IDH2* mutations, 1- and 2-year OS in patients with spliceosome mutations in the context of co-occurring *IDH1/2* mutations, was 92% and 83%, respectively. Interactions within this co-mutational subgroup were further explored. *IDH1/2* mutations were enriched in patients with *SRSF2* mutations compared with the entire cohort (42% vs 16%, $P = .01$). *IDH2* mutations specifically were enriched in patients with *SRSF2* mutations compared with the entire cohort ($P < .01$; Figure 2B). *SRSF2/IDH1/2*-mutated patients had 1- and 2-year OS rates of 100% and 88%, respectively (Figure 4D). *SRSF2/IDH1/2*-mutated patients had improved survival compared with *SRSF2* mutated patients without co-occurring *IDH* mutations (median OS, NR vs 8 months; FDR-adjusted $P = .02$), and demonstrated a slight trend toward improved survival compared with wild-type *IDH1/2*-mutated patients without *SRSF2* mutations (median OS, NR vs 22 months; FDR-adjusted $P = .098$). In patients with co-mutated *SRSF2* and *IDH2* with available NGS data for VAF analysis (n = 8), median VAF was most similar between *SRSF2* and *IDH2* mutations (42% and 41%, respectively), suggesting *SRSF2* and *IDH2* mutations may develop at similar points in time in the molecular hierarchy of AML (Figure 1C). *RAS* mutations were enriched in patients with *U2AF1* mutations and associated with numerically lower CRc (and MRD⁻ CR) rates. However, compared with wild-type patients with *RAS* mutations, no significant difference in survival was observed (median OS, 14 vs 13 months; $P = .68$; supplemental Figure 6).

Given the small numbers in our cohort, we also analyzed data from 892 samples obtained from 762 patients with AML from 2 previous studies^{19,20} to determine if the observed association between *SRSF2* and *IDH2* mutations in our cohort was retained in larger analyses. Although the frequency of identified *SRSF2* mutations was lower (n = 65, 9%), *IDH2* mutations were the most common

Table 3. Overall survival

Demographic	Event-free survival (95% CI), mo	Overall survival (95% CI), mo	P*
All patients (N = 119)	10 (9-15)	14 (12-NR)	—
Spliceosome (n = 39)	10 (6-NR)	35 (13-NR)	.58
ELN risk group			
Favorable	NR (3-NA)	NR (3-NA)	.48
Intermediate	10 (8-NA)	NR (13-NA)	.97
Adverse	7 (3-NA)	14 (5-NA)	.29
Cytogenetic group			
Diploid	NR (10-NA)	NR (—)	.57
Other intermediate	17 (9-NA)	35 (13-NA)	.40
Complex/adverse	3 (2-NA)	5 (3-NA)	.29
AML type			
De novo AML	18 (8-NA)	NR (—)	.35
Secondary AML	7 (6-NA)	10 (6-NA)	.80
Therapy-related AML	6 (5-NA)	8 (6-NA)	.69
Wild-type (n = 80)	11 (9-19)	14 (10-NA)	
ELN risk group			
Favorable	31 (13-NA)	NR (17-NA)	—
Intermediate	32 (7-NA)	NR (12-NA)	—
Adverse	9 (5.0-NA)	10 (8-18)	—
Cytogenetic group			
Diploid	31 (20-NA)	NR (—)	—
Intermediate	11 (7-NA)	19 (9-NA)	—
Complex/adverse	5 (5-10)	9 (6-12)	—
AML type			
De novo AML	19 (11-NA)	22 (14-NA)	—
Secondary AML	5 (3-NA)	9 (6-NA)	—
Therapy-related AML	6 (5.0-10)	10 (5-NA)	—

NA, not available.

*P value presented is comparison between spliceosome and wild-type cohorts for the respective demographic.

identified co-mutation, were significantly enriched in patients with *SRSF2* mutations (35%, $P < .01$) and were associated with improved survival compared with those with isolated *SRSF2* mutations (20 vs 10 months, $P = .02$) treated with standard cytotoxic chemotherapy.

Discussion

Mutations within genes encoding the spliceosome complex (*SRSF2*, *U2AF1*, *SF3B1*, *ZRSR2*) historically have defined a high-risk AML subgroup associated with inferior outcomes with intensive chemotherapy.⁶ In this analysis of patients treated with HMA+VEN combinations, patients with spliceosome mutations had outcomes similar to a wild-type cohort of patients, suggesting HMA+VEN therapy may be an effective treatment regimen in this population. Patients with *SRSF2* mutations were enriched for *IDH2* mutations and appeared to particularly benefit from VEN-based treatment. Co-mutations in cohesin complex and spliceosome genes resulted in an 80% 1-year OS rate; however, this small molecular subgroup necessitates follow-up in larger cohorts.

Molecular classification is standard in the diagnostic workup of AML both for its prognostic value and to guide treatment in an era of molecularly targeted therapies.^{14,21} Spliceosome mutations are frequently identified in older patients with myeloid malignancies, a group more likely to be ineligible for intensive chemotherapy.^{4,6} In this cohort, spliceosome mutations were identified at a frequency of ~33% in an older (median age, 75 years) population, consistent with prior analyses.⁶ Similar to prior investigations, patients with sAML had a numerically higher observed frequency of spliceosome mutations.^{2,5} Spliceosome-mutated patients demonstrated a heterogeneous molecular landscape, yet with a similar mutational burden compared with wild-type patients.⁵ Neither age nor mutation burden influenced OS with HMA+VEN treatment.

Molecular determinates of response to HMA+VEN have been reported.⁹ However, to our knowledge, the influence of co-occurring mutations in patients with spliceosome mutations has yet to be evaluated. In keeping with prior analyses, adverse cytogenetics and *TP53* mutations associated with inferior OS, whereas *NPM1* and *IDH1/2* mutations associated with favorable outcomes in both spliceosome and wild-type cohorts.

Recent investigations have identified a differential prognostic impact imparted by specific spliceosome mutations and resultant amino acid residue changes occurring within the same gene,^{16,17} and identified mutant *SF3B1* AML as a marker of VEN resistance.²² Although the small sample size precluded analysis of the differential impact of survival in patients with *SF3B1* K666 vs K700 mutations, the median OS of 35 months in this patient population warrants further investigation to determine if HMA+VEN may overcome VEN resistance observed in *SF3B1* mutated AML, and negate the negative impact associated with K666 mutations. The majority of *SF3B1*-mutated patients received decitabine in combination with VEN. Because *SF3B1*-mutated AML appears particularly sensitive to decitabine-based therapy,²³ the reduction in VAF from diagnosis to remission (median VAF at diagnosis 33% vs remission 6%) may represent retained sensitivity to hypomethylating agents or synergistic activity of HMA+VEN within this molecular subgroup. *SF3B1*-mutated patients had lower rates of MRD-negative CRc compared with *SRSF2* mutated patients, suggesting variable sensitivity to VEN between spliceosome mutations.

The enrichment of *RAS* mutations identified in patients with mutated *U2AF1* appears consistent across studies.¹⁸ Because signaling mutations are a known primary and adaptive resistance mechanism to VEN,⁹ it is plausible the poor responses observed in these patients is in part attributable to the influence of *RAS* mutations as opposed to *U2AF1* itself, resulting in increased resistance (no *U2AF1* patients achieved an MRD-negative CR) and relapse to HMA+VEN-based therapy.

SRSF2 mutations have demonstrated sensitivity to VEN-based therapies.^{10,11} Within the spliceosome cohort, *IDH2* mutations were enriched in patients with *SRSF2* mutations, and associated with excellent 1- and 2-year OS (100% and 87%, respectively). Analysis of genomic data from larger independent patient cohorts confirmed this positive association.^{19,20} Single-cell DNA analysis of AML additionally demonstrated *SRSF2/IDH2* frequently co-occur within the same leukemic clone,²⁴ with the mutational pair

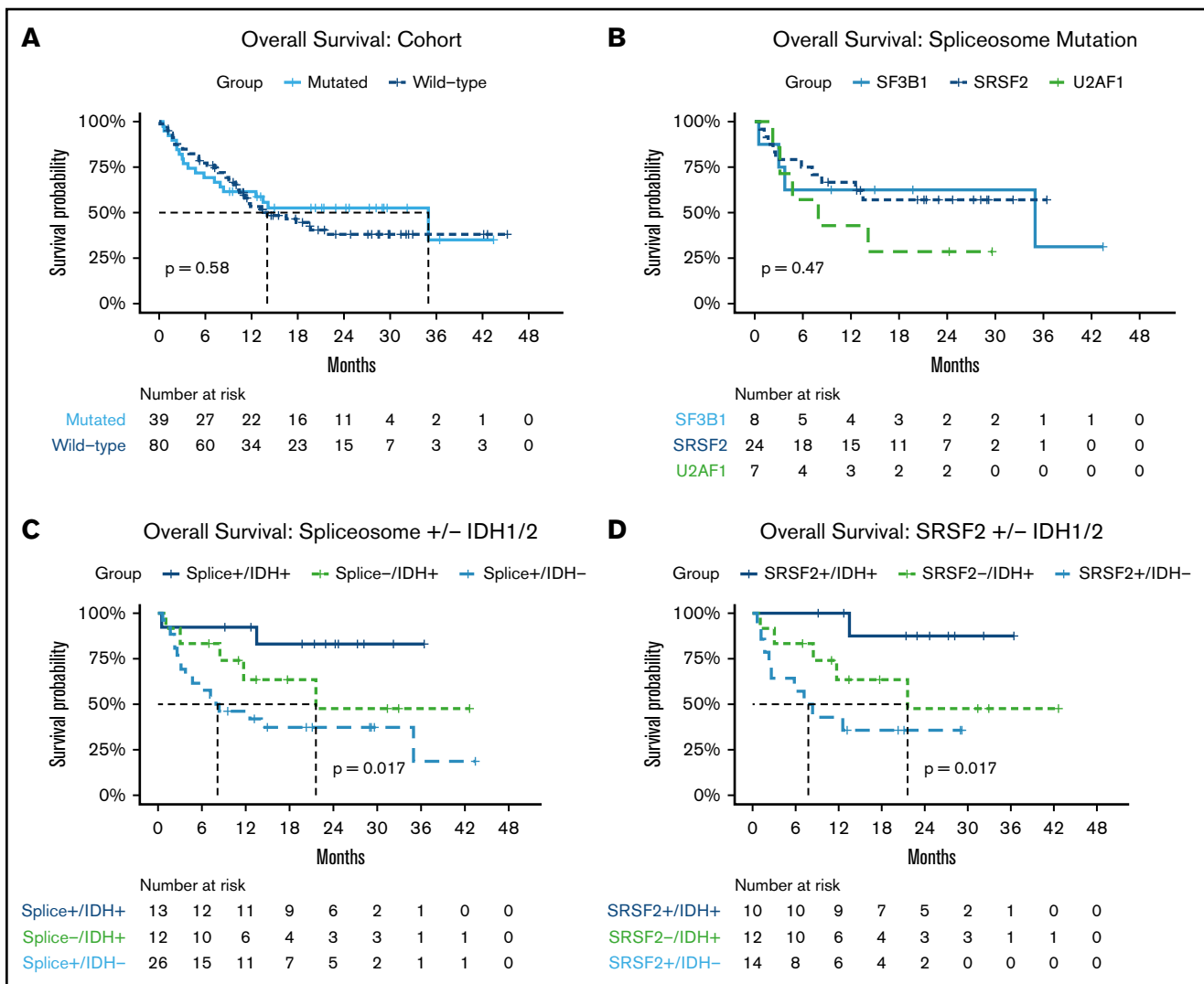


Figure 4. Survival outcomes by cohort and mutational subgroups. Overall survival in (A) patients with vs without spliceosome mutations; (B) by specific spliceosome mutation; (C) in patients with co-occurring spliceosome and *IDH1/2* mutations, wild-type patients with *IDH1/2* mutations, and spliceosome patients without *IDH1/2* mutations; and (D) in patients with co-occurring *SRSF2* and *IDH1/2* mutations, wild-type patients with *IDH1/2* mutations, and *SRSF2*-mutated patients without *IDH1/2* mutations.

implicated in leukemogenesis.²⁵ Although limited by sample size, bulk NGS data found similar VAF frequencies of *IDH2* and *SRSF2*, suggesting these mutations develop at similar time points in leukemic clonal evolution. This observation is further supported through the identification of persistent co-occurring *SRSF2* and *IDH2* mutations in 2 patients with MRD⁻ remissions by FC. Prior work has highlighted the risk of leukemic progression imparted by spliceosome and *IDH* mutations when identified as components of clonal hematopoiesis, particularly within an older patient population.^{26,27} Thus, the persistence or emergence of mutations in remission representing preleukemic or novel leukemic clones highlights the importance of the proper identification and discrimination of such mutations from residual leukemia through expert hematopathological interpretation and incorporation of alternative methods of MRD assessment (ie, FC).

A clear explanation for the favorable outcomes in *SRSF2/IDH2*-mutated patients is not readily apparent; however, spliceosome

mutations may influence BCL-2 family expression, and in turn modulate responses to VEN-based therapy.²⁸ In our cohort, the improved survival in *SRSF2* mutated patients treated with HMA+VEN appeared largely driven by the co-occurrence of *IDH1/2* mutations with favorable survival to that seen in historic cohorts of *SRSF2/IDH1/2*-mutated patients treated with conventional chemotherapy. Survival in *SRSF2*-mutated patients without *IDH* mutations was a modest 8 months, a discordant finding from prior analyses.¹¹ Although median follow-up time was adequate (24 months), these results must nonetheless be interpreted in the context of the small cohort sizes and retrospective nature of this analysis. Future investigations including larger patient cohorts are warranted to confirm these findings.

In conclusion, we demonstrate outcomes in patients with spliceosome mutations treated with HMA+VEN are comparable to those seen in wild-type patients. HMA+VEN therapy may overcome some of the adverse prognosis associated with specific spliceosome

mutations or spliceosome mutations in the context of standard cytotoxic chemotherapy, with certain mutational pairs (*SRSF2/IDH2*) defining molecular groups imparting a favorable prognostic impact. Alternatively, other mutational pairs (*U2AF1/RAS*) may identify patients with suboptimal responses to HMA+VEN. Confirmation of these findings in prospective cohorts using molecularly targeted therapies will aid in the refinement of molecular subgroups of patients with increased sensitivity and response to venetoclax-based regimens.

Authorship

Contribution: C.A.L., C.D.D., and S.L. performed data collection and analysis and prepared the manuscript; K.F. performed data analysis and assisted in manuscript preparation; S.P. performed data collection; and C.D.D., S.L., G.M.-B., A.M., T.K., N.D., G.B., N.P., K.S., Y.A., M.Y., N.J.S., K.C., M.O., K.P.P., E.J., F.R., H.M.K., G.G.-M., K.T., and M.Y.K. assisted in patient care, manuscript preparation, and review.

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