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Authors

Richardson, Daniel
Swoboda, David
Moore, Dominic
et al.

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Genomic characteristics and prognostic significance of co-mutated *ASXL1/SRSF2* acute myeloid leukemia

Daniel R. Richardson[1,2,3], **David M. Swoboda**[4], **Dominic T. Moore**[1], **Steven M. Johnson**[5], **Onyee Chan**[4], **Jonathan Galeotti**[5], **Sonia Esparza**[1,2], **Mohammad O. Hussaini**[4], **Hendrick Van Deventer**[1,2], **Matthew C. Foster**[1,2], **Catherine C. Coombs**[1,2], **Nathan D. Montgomery**[1,5], **David A. Sallman**[4], **Joshua F. Zeidner**[1,2]

[1]Lineberger Comprehensive Cancer Center, The University of North Carolina School of Medicine, Chapel Hill, North Carolina

[2]Division of Hematology, Department of Medicine, The University of North Carolina School of Medicine, Chapel Hill, North Carolina

[3]The Cecil G. Sheps Center for Health Services Research, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina

[4]Department of Malignant Hematology, H. Lee Moffitt Cancer Center and Research Institute, Tampa, Florida

[5]Department of Pathology and Laboratory Medicine, The University of North Carolina School of Medicine, Chapel Hill, North Carolina

Abstract

The *ASXL1* and *SRSF2* mutations in AML are frequently found in patients with preexisting myeloid malignancies and are individually associated with poor outcomes. In this multi-institutional retrospective analysis, we assessed the genetic features and clinical outcomes of 43 patients with $ASXL1^{mut}SRSF2^{mut}$ AML and compared outcomes to patients with either *ASXL1* (n = 57) or *SRSF2* (n = 70) mutations. Twenty-six (60%) had secondary-AML (s-AML). Variant allele fractions suggested that *SRSF2* mutations preceded *ASXL1* mutational events. Median overall survival (OS) was 7.0 months (95% CI:3.8,15.3) and was significantly longer in patients with de novo vs s-AML (15.3 vs 6.4 months, respectively; $P = .04$ on adjusted analysis). Compared to $ASXL1^{mut}SRSF2^{wt}$ and $ASXL1^{wt}SRSF2^{mut}$, co-mutated patients had a 1.4 and 1.6 times increase in the probability of death, respectively ($P = .049$), with a trend towards inferior OS (median OS = 7.0 vs 11.5 vs 10.9 months, respectively; $P = .10$). Multivariable analysis suggests this difference in OS is attributable to the high proportion of s-AML patients in the co-mutated cohort (60% vs 32% and 23%, respectively). Although this study is limited by the retrospective data collection and the relatively small sample size, these data suggest that $ASXL1^{mut}SRSF2^{mut}$ AML is a distinct subgroup of AML frequently associated with s-AML and differs from $ASXL1^{mut}SRSF2^{wt}$/$ASXL1^{wt}SRSF2^{mut}$ with respect to etiology and leukemogenesis.

Correspondence: Joshua F. Zeidner, 170 Manning Dr. CB #7305, Chapel Hill, NC 27599. joshua_zeidner@med.unc.edu.
Daniel R. Richardson, David M. Swoboda, David A. Sallman, and Joshua F. Zeidner contributed equally to this study.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

1 | INTRODUCTION

Acute myeloid leukemia (AML) is a hematologic malignancy of myeloid progenitors resulting in compromised hematopoiesis and bone marrow failure.[1] Diverse and complex genetic alterations result in highly heterogeneous outcomes for patients. Prognosis has traditionally been determined using clinical characteristics and cytogenetic abnormalities. However, the recent widespread adoption of next generation sequencing (NGS) has allowed for the identification of several prognostically distinct genomic subgroups.[2–6] The largest genomic classification of AML patients by Papaemmanuil et al. proposed 11 distinct subgroups based on patterns of leukemia-initiating mutations, comutations and genomic heterogeneity, which are hypothesized to better define the disease biology and predict outcomes.[4]

One proposed subgroup is defined by mutations in chromatin modifying and/or RNA-splicing genes.[4] This chromatin-spliceosome subgroup includes patients with concomitant mutations in the chromatin modifying gene, *ASXL1*, and the splicesosome component, *SRSF2*, (*ASXL1*^{mut}*SRSF2*^{mut} AML). In the 15 patients with *ASXL1*^{mut}*SRSF2*^{mut} AML reported in this analysis, overall survival (OS) was dismal with no long-term survivors suggesting that these mutations may have multiplicative adverse effects.[4] The small number of co-mutated (*ASXL1*^{mut}*SRSF2*^{mut}) patients in this study precluded the ability to determine how clinical factors such as type of treatment or a prior history of myeloid neoplasm influenced outcomes. Harboring an *ASXL1* mutation, regardless of the presence or absence of a mutation in *SRSF2*, is a known poor prognostic indicator.[3,4,7–10] *SRSF2* is associated with poor outcomes for MDS patients, though the prognostic significance in AML is less well-defined.[11–14] Determining the effect of a prior myeloid malignancy is of particular importance because *ASXL1* and *SRSF2* mutations are both common in myelodysplastic syndrome (MDS)[15] and chronic myelomonocytic leukemia (CMML).[16] We recently reported that *ASXL1*^{mut}*SRSF2*^{mut} AML, in contrast to *ASXL1*^{mut}*SRSF2*^{wt} AML, shares a mutational profile and immunophenotype with CMML suggesting that this genomic profile may identify patients with secondary AML (s-AML) from preexisting CMML.[17] Further, Lindsley and colleagues identified *ASXL1* and *SRSF2*, along with six other mutations related to chromatin and RNA-splicing (*EZH2*, *BCOR*, *STAG2*, *SF3B1*, *U2AF1*, and *ZRSR2*), to be highly specific (>95%) for predicting s-AML, an etiologic subtype of AML known to confer very poor outcomes..[18,19]

In this multi-institutional retrospective analysis, we sought to assess the genetic features and analyze the clinical outcomes of a larger cohort of patients with *ASXL1*^{mut}*SRSF2*^{mut} AML, and compare survival outcomes of this cohort to patients with *ASXL1*^{mut}*SRSF2*^{wt} AML and *ASXL1*^{wt}*SRSF2*^{mut} AML. We hypothesized that *ASXL1*^{mut}*SRSF2*^{mut} AML would be associated with worse outcomes and may represent a unique genomic footprint of s-AML irrespective of whether patients were diagnosed with a preexisting myeloid neoplasm.

2 | METHODS

2.1 | Study design

This is a multi-institutional retrospective cohort study involving patients who were identified through the electronic medical record review at the University of North Carolina in Chapel Hill, NC (UNC) and Moffitt Cancer Center in Tampa, FL (Moffitt) from 2011 to 2020. Patients were included in all analyses if they were ≥ 18 years old and had newly diagnosed AML with *ASXL1* or *SRSF2* mutations identified by NGS from 2011 to 2020. The 2016 WHO guidelines were used as diagnostic criteria for AML.[6] In the primary analysis, outcomes of $ASXL1^{mut}SRSF2^{mut}$ AML were described to evaluate the association between co-variates including etiology (de novo, s-AML, treatment-related AML [t-AML]) and survival. In a secondary analysis, survival outcomes of $ASXL1^{mut}SRSF2^{mut}$ patients were compared to patients with $ASXL1^{mut}SRSF2^{wt}$ AML and $ASXL1^{wt}SRSF2^{mut}$ AML to evaluate the differences between these populations. This study was approved by the institutional review board at UNC and Moffitt according to the declaration of Helsinki.

2.2 | Next-generation sequencing

Next generation sequencing was performed on DNA collected from diagnostic bone marrow or peripheral blood. Table S1 outlines the sequencing panels used for each patient with $ASXL1^{mut}SRSF2^{mut}$ AML. A threshold of ≥5% variant allele fraction (VAF) for individual gene mutations was considered positive for most variants. However, for samples analyzed using the Illumina TruSight Myeloid 54-gene panel, *ASXL1* c.1934dupG (p.Gly646fs) mutations required a minimum 10% VAF, given known potential false positive results at lower VAF on this platform.[20]

To be included in this study, patients were required to have *ASXL1* or *SRSF2* variants defined as pathogenic/likely pathogenic by the reporting laboratory. Germline control samples were not sequenced in parallel, but variants with high population allele frequencies in any subpopulation in the genome aggregation database (gnomAD, https://gnomad.broadinstitute.org) were excluded as presumed benign germline variants.

2.3 | Outcomes

The primary outcome was OS defined as time from diagnosis of AML to date of death or end of the study period (February 28, 2020). Patients who were alive at this time were censored. For patients with $ASXL1^{mut}SRSF2^{mut}$ AML, the primary exposure was s-AML. S-AML was defined as having a documented history of a previous myeloid neoplasm. T-AML was defined as developing AML after receiving cytotoxic chemotherapy or radiation. Patients who developed a treatment-related myeloid neoplasm prior to the development of AML were classified as s-AML. Patients who did not have s-AML or t-AML were classified as having *de novo* AML. Both MDS and myeloproliferative neoplasms (MPNs) were defined according to current WHO criteria. The secondary outcome was rate of complete remission (CR) or complete remission with incomplete hematologic recovery (CRi) following initial chemotherapy defined by ELN guidelines.[3] Exploratory analyses evaluated associations between treatment intensity, as well as serologic, genomic, and cytogenetic factors and outcomes. Patients receiving azacitidine or decitabine, regardless of

the dose or schedule, were classified as receiving hypomethylating agents (HMAs). Patients receiving anthracycline-based chemotherapy (daunorubicin, idarubicin, or mitoxantrone) were considered to have received intensive induction chemotherapy (IC). Investigational agents given to patients as part of a clinical trial in addition to these regimens were not considered when determining treatment intensity.

2.4 | Covariates

Covariates considered were based on literature review and included site, age, gender, race, performance status, cytogenetic risk group, splenomegaly, number of mutations, variant allele fraction of *SRSF2* and *ASXL1*, and white blood cell count, hemoglobin, platelet count, and lactate dehydrogenase (LDH) at diagnosis.

2.5 | Statistical methods

Patients' baseline characteristics were summarized using descriptive statistics. Fisher's exact test was used to compare categorical variables and the Wilcoxon two-group test and the Kruskal-Wallis test were used for to compare groupings of continuous variables. The Kaplan–Meier method was used to estimate the time-to-event function of OS. OS was calculated using the time from initial diagnosis (either by bone marrow biopsy or, if unavailable, flow cytometry of peripheral blood) to death or date of last contact (censored). Cox regression modeling was used to evaluate the association of OS and select patient covariates. Both univariable and adequately powered multivariable models were investigated. The OS curves were compared using log-rank and score tests. Logistic regression models were used to examine the association of select covariates to the dichotomized outcome response variable of CR/CRi to not CR/CRi. All reported P values are two-sided with P values less than .05 considered significant. Statistical analyses were done using Stata (Version 16.1, StataCorp, College Station, TX), SAS (version 9.4, Cary, NC) or R (2019, R Foundation, https://www.R-project.org).

3 | RESULTS

3.1 | Patient characteristics

Of 1564 AML patients screened, 43 (2.7%) were found to have $ASXL1^{mut}SRSF2^{mut}$ AML. Baseline characteristics of the $ASXL1^{mut}SRSF2^{mut}$ cohort are included in Table 1. The median age of patients was 71 years; most patients (81%) were white. Twenty-eight (64%) had normal cytogenetics; 35 (81%) had intermediate-risk cytogenetics by current ELN guidelines. Twenty-six patients (60%) were classified as having s-AML arising from MDS (n = 17), CMML (n = 7), and MPNs (n = 2) including one patient who developed t-MN prior to developing AML. Sixteen (37%) patients were classified as having *de novo* AML. One patient was classified as t-AML. Patients with s-AML had fewer blasts in the bone marrow as compared to *de novo* patients (median 30% vs 64%, $P < .001$).

3.2 | Mutations and variant allele fraction

Figure 1 illustrates the identified co-mutations and clinical response to initial treatment among $ASXL1^{mut}SRSF2^{mut}$ AML patients. The most common additional co-mutations seen in this cohort were *TET2* (49%), *RUNX1* (35%), *STAG2* (25%), *IDH1* (16%), *NRAS* (14%)

and *SETBP1* (14%). Although the small sample size precluded statistical analyses, *IDH1*, *RUNX1*, *JAK2*, *NPM1* and *EZH2* mutations appeared to be more common in de novo AML whereas *ETV6*, *CEBPA* and *STAG2* mutations appeared to be more common in s-AML (Table S2).

Among co-mutated patients, all (43/43) *ASXL1* variants resulted in protein truncation. In 29 cases, the pathogenic *ASXL1* mutation was an insertion/deletion resulting in reading frameshift, and in 14 cases, the pathogenic *ASXL1* mutation was a single nucleotide nonsense variant that introduced an early termination codon. Consistent with prior publications and public databases, *ASXL1* c.1934dupG (p. Gly646TrpfsTer12) was the most common *ASXL1* mutation, occurring in 21 of 43 cases (49%). All 43 *SRSF2* mutations impacted codon 95, the canonical hotspot for mutations in this gene. Missense mutations impacting codon 95 were present in 40 cases (91%), with the four remaining variants (9%) representing in-frame deletions at this site. A list of all reported variants is included in Table S3.

The *SRSF2* mutations were consistently found at a high variant allele fraction (VAF), typically close to 50% (range 12% - 61%, median 45%), suggesting a heterozygous driver mutation present in most cases. In contrast, the VAF of *ASXL1* mutations was more variable (range 6% – 49%, median 34%). To infer mutation ontogeny, *SRSF2* and *ASXL1* VAFs were compared by calculating a ratio of *SRSF2* VAF: *ASXL1* VAF. In 36/43 (84%) patients, the ratio was ≥1.0, suggesting that *SRSF2* is the earlier mutational event in the majority of cases (Figure 2). Patients with s-AML had a non-significant increase in *SRSF2*:*ASXL1* VAF compared with de novo AML ($P = .10$).

3.3 | Overall survival

The median follow-up for $ASXL1^{mut}SRSF2^{mut}$ AML survivors was 11.2 months. The median OS was 7.0 months (95% CI: 3.8, 15.3; Figure 3(A)). Median OS for patients with s-AML (n = 26) and de novo AML (n = 16) was 6.4 months (95% CI: 2.5, 11.2) and 15.3 months (95% CI: 3.6, 24.2, $P = .09$), respectively (Figure 3(B)). The patient with t-AML survived 7 days. Median OS did not differ by age ($P = .16$) or total number of mutations (continuous, $P = .40$). Note, SWOG cytogenetic risk, splenomegaly, lLDH, hemoglobin, white blood cell count, blast count at diagnosis, type of secondary AML (MDS vs MPN vs CMML) and VAFs of *ASXL1* or *SRSF2* mutations were also not significantly associated with OS. Although site was significantly associated with OS on a univariable model ($P = .04$), adjusting for patients who did not receive treatment eliminated the significance of this association demonstrating that this association was due to variation in patient acuity. After adjusting for site on multivariable analysis, s-AML was significantly associated with having worse OS ($P = .04$) as compared to *de novo* AML. Adding additional covariates to the multivariable model did not significantly change this association, nor improve model fit.

Thirty-six patients received either IC (n = 20) or an HMA (n = 16) (Table S4). OS varied significantly by receipt of chemotherapy (no treatment vs IC vs HMAs, $P < .001$, Figure 3(C), Table S4) mostly due to the very poor outcomes of the seven patients who received no chemotherapy. Median OS for these patients was only 1.6 months compared to 10.6 months for those who received chemotherapy ($P < .001$). As compared to those who received HMAs

(n = 16), patients who received IC (n = 20) appeared to have a longer median OS although this was not statistically significant (15.3 months vs 6.6 months, $P = .20$). Patients achieving a CR or CRi (n = 11) on first induction had a median OS of 15.3 months (95% CI: 5.3, 24.2). Six patients (four *de novo* and two s-AML) underwent allogeneic stem cell transplant (allo-SCT) with a median OS not reached (median follow-up 15.6 months). Five of the six (83%) received IC as initial treatment; four (67%) were alive at last follow-up including the two patients with s-AML.

Although not statistically significant, the rates of CR/CRi were higher following IC (8/20: 40%) compared with HMAs (3/16: 19%, $P = .17$). Of the 16 patients with *de novo* AML, seven achieved CR/CRi (44%): five of nine patients receiving IC (56%) and two of seven patients receiving HMAs (28%). Of the 26 patients with s-AML, four achieved CR/CRi (15%): three of 11 patients receiving IC (27%) and one of 10 patients receiving HMAs (10%).

Notably, two co-mutated patients had *NPM1* mutations, both of whom had a normal karyotype and were classified as de novo AML. Both received IC, achieved a CR/CRi, and did not undergo allo-SCT. They are both alive without relapse at over 2 years follow-up.

3.4 | OS comparison among co-mutated and *ASXL1*^{mut}*SRSF2*^{wt} *and ASXL1*^{wt}*SRSF2*^{mut} patients

Fifty-seven patients were identified with *ASXL1*^{mut}*SRSF2*^{wt} AML and 70 patients were identified with *ASXL1*^{wt}*SRSF2*^{mut} AML. The median age of patients did not differ between co-mutated patients (70.3 years) and *ASXL1*^{mut}*SRSF2*^{wt} (69.5 years) or *ASXL1*^{wt}*SRSF2*^{mut} patients (70.2 years, $P = .95$). Race, gender, and performance status also did not differ among the groups. Co-mutated patients were more likely to be classified as s-AML (60% vs 32% vs 23%, $P = .001$). Patients in the *ASXL1*^{mut}*SRSF2*^{wt} and *ASXL1*^{wt}*SRSF2*^{mut} subgroups had roughly equivalent probability of death. However, co-mutated patients had a 1.4 times increase in the probability of death compared to *ASXL1*^{mut}*SRSF2*^{wt} patients, and 1.6 times the probability of death when compared to *ASXL1*^{wt}*SRSF2*^{mut} patients ($P = .049$). Co-mutated patients appeared to have worse median OS compared to *ASXL1*^{mut}*SRSF2*^{wt} and *ASXL1*^{wt}*SRSF2*^{mut} patients (7.0 months, [95% CI 3.8, 15.3] vs 11.5 months [95% CI 7.8, 16.4] vs 10.9 months [95% CI 8.1, 19.4], respectively, $P = .10$; Figure 3(D)), though this did not reach statistical significance. The multivariable model for OS using s-AML status alone was significantly better than the model using mutation status alone ($P = .001$) suggesting that the difference in survival outcomes is due to differences in the proportion of s-AML patients. The OS by mutational status and etiology is shown in Table S5.

4 | DISCUSSION

Over the last decade, advances in the understanding of the genetic determinants of AML and the widespread adoption of NGS in routine care has allowed for refinement of prognostically distinct genetic subgroups. Here, we report the largest cohort to date of patients with *ASXL1*^{mut}*SRSF2*^{mut} AML and compare survival outcomes to patients with either *ASXL1* or *SRSF2* mutations. The overall prevalence of *ASXL1*^{mut}*SRSF2*^{mut} AML was 2.7% which is consistent with data available from The Cancer Genome Atlas (13/672, 1.9%).[21]

The cohort of patients with *ASXL1*^{mut}*SRSF2*^{mut} AML was enriched for s-AML (60%) and harbored a relatively high number of overall mutations (median = 4). Although comparisons of number of mutations across datasets are prone to bias due to differential effects of sensitivity and breadth of sequencing, the high number of mutations illustrates the complexity of the mutational ontogeny in these patients. Other concomitant mutations frequently seen in this cohort of patients include those associated with preexisting myeloid neoplasms (such as *RUNX1*, *STAG2*, *NRAS*, *SETBP1*, and *CBL*) supporting the work of others suggesting that this genomic profile may be a footprint for preexisting MDS/MPNs.[18] In most patients (84%), *SRSF2* VAF was higher than *ASXL1* VAF, suggesting that these patients had a heterozygous *SRSF2* driver mutation and that *SRSF2* mutations preceded *ASXL1* mutational events. The ratio of *SRSF2* to *ASXL1* appeared higher among s-AML patients ($P = .10$) which might suggest differences in leukemogenesis between groups though larger studies are needed to validate this result.

Both *ASXL1* and *SRSF2* mutations are common in MDS and CMML and are frequently found in patients with s-AML.[9,13,15] Our group has previously shown that *ASXL1*^{mut}*SRSF2*^{mut} AML has mutational and immunophenotypic features overlapping with CMML.[17] Thus, we hypothesized those patients who are classified as having *de novo ASXL1*^{mut}*SRSF2*^{mut} AML may have actually harbored a preexisting undiagnosed myeloid neoplasm, and are biologically and clinically similar to patients with documented s-AML. Our findings confirm that *ASXL1*^{mut}*SRSF2*^{mut} AML patients have a poor prognosis (median OS: 7.0 months), consistent with recent studies.[4, 22] This poor prognosis was shared among patients regardless of age, number of mutations, or cytogenetic risk category, which are typically strong predictors of OS. However, OS was worse in patients with s-AML when compared with *de novo* AML (6.1 months vs 15.4 months), after adjusting using multivariable analysis. While this does not disprove our hypothesis as larger samples are needed to confidently control for multiple covariates, it does highlight the continued clinical importance of a preexisting myeloid neoplasm. Additional studies, such as single cell sequencing, are needed to definitively determine whether clinical *de novo* *ASXL1*^{mut}*SRSF2*^{mut} AML biologically differs from *ASXL1*^{mut}*SRSF2*^{mut} s-AML and to describe the outcome of these clones after different treatment regimens.

Given our initial hypothesis, we speculated that characteristics and outcomes of co-mutated patients would be different than *ASXL1*^{mut}*SRSF2*^{wt} patients. This is clinically relevant as there is an established association between *ASXL1* mutations and adverse-risk disease.(4, 7–10) Analysis of VAF ratios in our data suggest that, among co-mutated patients, *SRSF2* mutational events preceded *ASXL1* mutations. Together with our previous findings that co-mutated patients harbor a unique immunophenotype, and mutational profile similar to patients with CMML, and differing significantly from *ASXL1*^{mut}*SRSF2*^{wt} AML,[17] these data suggest that *ASXL1*^{mut}*SRSF2*^{mut} AML is a unique subgroup of AML with respect to etiology and leukemogenesis as compared to *ASXL1*^{mut}*SRSF2*^{wt} AML. Survival outcomes also appeared to differ between groups with worse outcomes in co-mutated patients (Figure 3(D)). Although we demonstrate that survival differences are attributable to the larger proportion of s-AML patients in the co-mutated group, the striking discrepancy in proportion of s-AML patients (60% vs 32%) serves only to further illustrate the difference between *ASXL1*^{mut}*SRSF2*^{mut} AML and *ASXL1*^{mut}*SRSF2*^{wt} AML.

There have been substantial therapeutic advances in the treatment of AML since the inception of this data. Liposomal cytarabine and daunorubicin (CPX-351) was FDA-approved for AML with myelodysplasia-related changes (MRC) in 2017, and venetoclax was FDA-approved in addition to HMAs or low dose cytarabine in 2018 for patients who are not candidates for IC.[23–26] Because *ASXL1*^{mut}*SRSF2*^{mut} AML is more prevalent in elderly patients and those with MRC, further studies are needed to investigate the use of these novel regimens in this population. Only two patients in this cohort received CPX-351 and no one received venetoclax. Monocytic phenotypes have been shown to up-regulate *MCL-1* and thus confer resistance to venetoclax-based regimens.[27] Further investigation is warranted to determine whether *ASXL1*^{mut}*SRSF2*^{mut} AML may have a less favorable response to venetoclax-based regimens due to its association with monocytic phenotypes.[17] Recent studies suggest that *ASXL1* mutations may predict for response to azacitidine and anti-PD-1 combinations.[28] However, there is a lack of data analyzing outcomes of patients with concomitant mutations in *ASXL1* and *SRSF2* receiving immune-based therapies. Given the relatively high mutational burden and poor outcomes with conventional chemotherapy regimens in this cohort, immune-based investigational strategies should be further explored in future trials.

The *NPM1* mutations are associated with de novo AML and typically confer a favorable response to therapy and improved clinical outcomes.[29–32] Some suggest that *NPM1* and *ASXL1* mutations may actually represent different routes of leukemogenesis given substantial differences in outcomes and other factors between groups.[33] In this cohort, two patients with *NPM1* mutations have survived over 2 years without allo-SCT or relapse suggesting that *NPM1* mutations may continue to confer favorable outcomes even with these concomitant pathogenic mutations. This is consistent with current ELN guidelines that recommend against classifying patients with concomitant *ASXL1* and *NPM1* as adverse-risk based on the presence of *ASXL1* alone.[3]

This study has several important limitations. First, while this is the largest cohort to date of *ASXL1*^{mut}*SRSF2*^{mut} AML, the small effective sample size limited our ability to detect statistically significant associations to only large effect sizes. This is particularly relevant in our comparison of survival outcomes between *de novo* vs. s-AML and IC vs. HMA therapy. Comparing outcomes by treatment regimen in a retrospective review is complicated by both known and unknown confounders. Although we attempted to account for known confounders through multivariable analysis, the small sample size limited exploratory models to only those with two or three covariates. Additionally, our data are taken from two large academic referral centers and are therefore susceptible to selection bias, which limits generalizability. We believe that the effect of this selection bias is minimal because most AML patients who are candidates for chemotherapy are treated at similar centers.

Nonetheless, these findings have several practical implications. First, although this study contributes to the body of evidence on the importance of *ASXL1*/*SRSF2* mutational status on prognosis, our findings highlight the critical importance of clinically-defining s-AML, especially for patients who are candidates for intensive therapies and allo-SCT. Though there are currently no FDA-approved agents that specifically target *ASXL1* or *SRSF2*, fit patients may be considered for early allo-SCT and/or clinical trials given poor outcomes

with conventional chemotherapy agents. Further, frail patients who are not eligible for intensive induction should be prioritized for novel approaches given dismal outcomes with HMA therapy. These data add to the accumulating evidence for utilizing NGS at diagnosis in AML to inform not only prognosis but also treatment regimen and intensity of therapy. Awaiting full molecular and cytogenetic results prior to initiating induction chemotherapy has been shown to be feasible and is currently being utilized in clinical trials such as BEAT AML to guide therapeutic decisions.[34] This strategy may become the standard of care as rapid NGS panels are becoming more available and more molecularly targeted agents are evaluated during induction.[35–37] Importantly, the results of this study need to be replicated with other data sets from different institutions and in different practice settings. Needless to say, integration of these data into risk models will be highly valuable. Given the poor prognosis seen among patients in this cohort, investigational trials with novel therapeutic agents are sorely needed.

In conclusion, we have provided further support to existing evidence for the classification of a unique subset of AML patients with co-mutated *ASXL1* and *SRSF2*. Eligible patients may benefit from IC and early allo-SCT though future studies are warranted to validate these data and identify preferred treatment regimens for this patient population.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

FUNDING INFORMATION

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CONFLICT OF INTEREST

D.R.R., D.M.S., D.T.M, S.M.J, O.C., J.G., S.E., H.V.D., and N.D.M report no conflicting financial interests. M.A.H has served as a consultant for Adaptive Technologies, Amgen, Decibio, Guidepoint, Stemline, Jannsen. M.C.F has received grant funding from Bellicum Pharmaceulticals, Celgene, and Macrogenomics; and has received consulting fees from Abbvie, Novartis, and Shire. C.C.C has served as a consultant for Abbvie and Covance; has received honoraria from Abbvie, AstraZeneca, LOXO, MEI Pharma, Octapharma; and has received institutional research funding from Gilead, Incyte, H3 Biomedicine, and LOXO. D.A.S. has served on the advisory board for Agios, BMS, Celyad, Intellia, Kite, Syndax; has been a consultant for Incyte; has received research funding from Celgene and Jazz; and has been on the speakers bureau for AbbVie, Agios, Incyte, and Novartis. J.F.Z has received honoraria from AbbVie, Agios, Bristol Myers Squibb/Celgene, Daiichi-Sankyo, Genentech, Pfizer, and Takeda; has received consultancy fees from AsystBio Laboratories, Celgene and Takeda; has received research funding from AROG, Celgene, Forty Seven, Merck, Sumitomo Dainippon Pharma, and Takeda.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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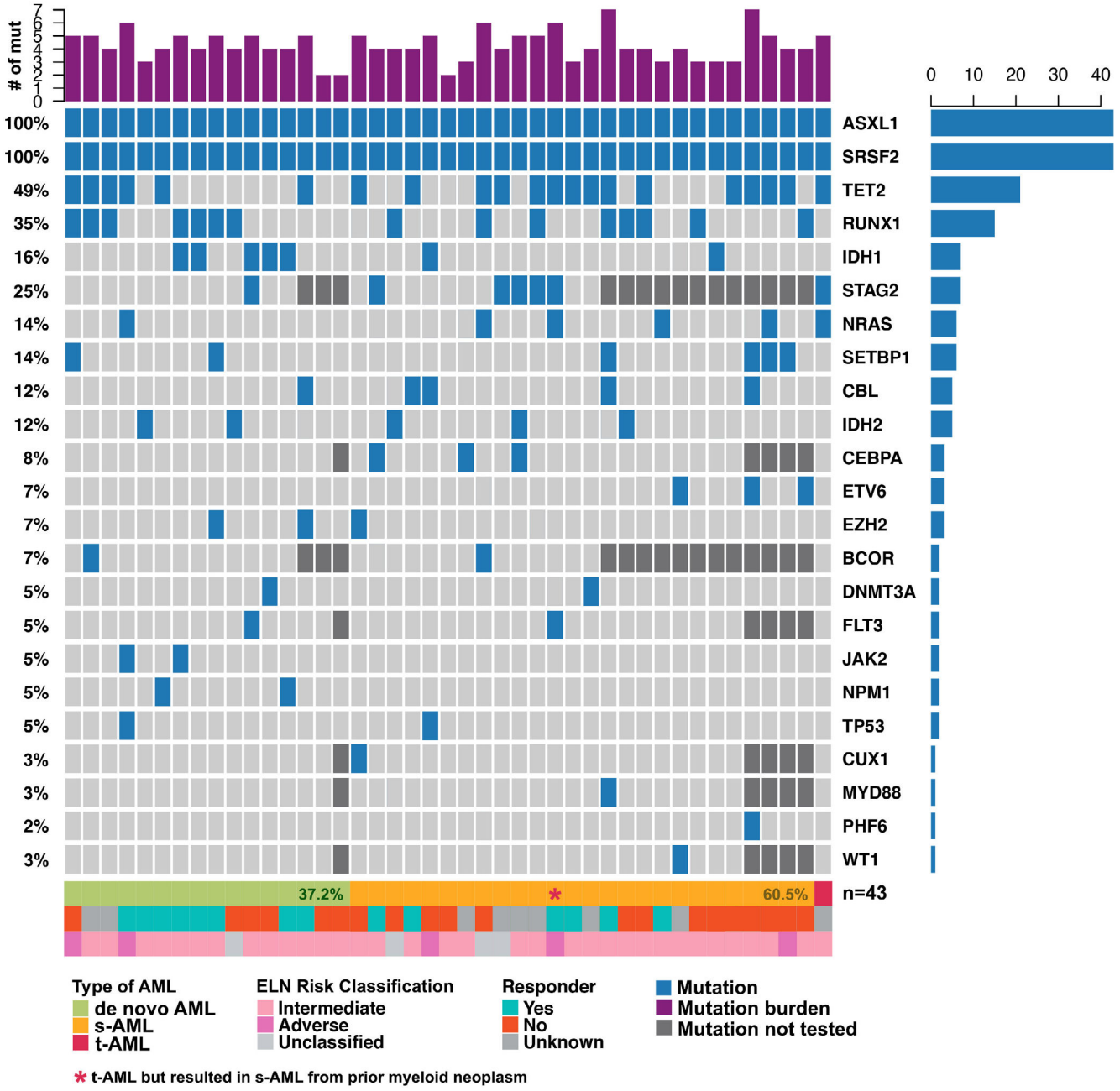

FIGURE 1.

Co-mutations, ontogeny and responses of patients with co-mutated *ASXL1/SRSF2*. Patients are sorted left-to-right by type of AML (de novo, secondary-AML [s-AML], or treatment-related AML [t-AML]). Number of mutations includes all unique mutations identified including *ASXL1* and *SRSF2*

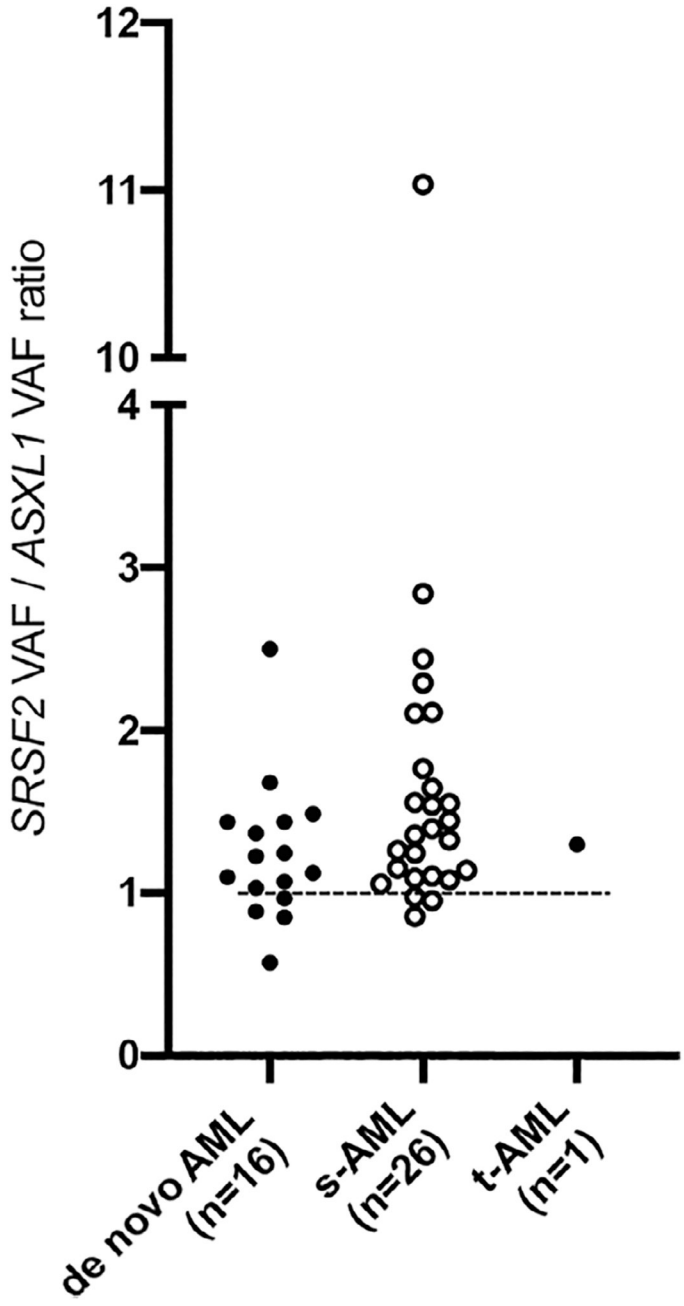

FIGURE 2.

Ratio of variant allele fraction (VAF) of *SRSF2* to *ASXL1*, stratified by de novo, secondary-AML (s-AML) and treatment-related AML (t-AML). Dashed line is one. Values above one indicate that the VAF of *SRSF2* is higher than the VAF of *ASXL1*

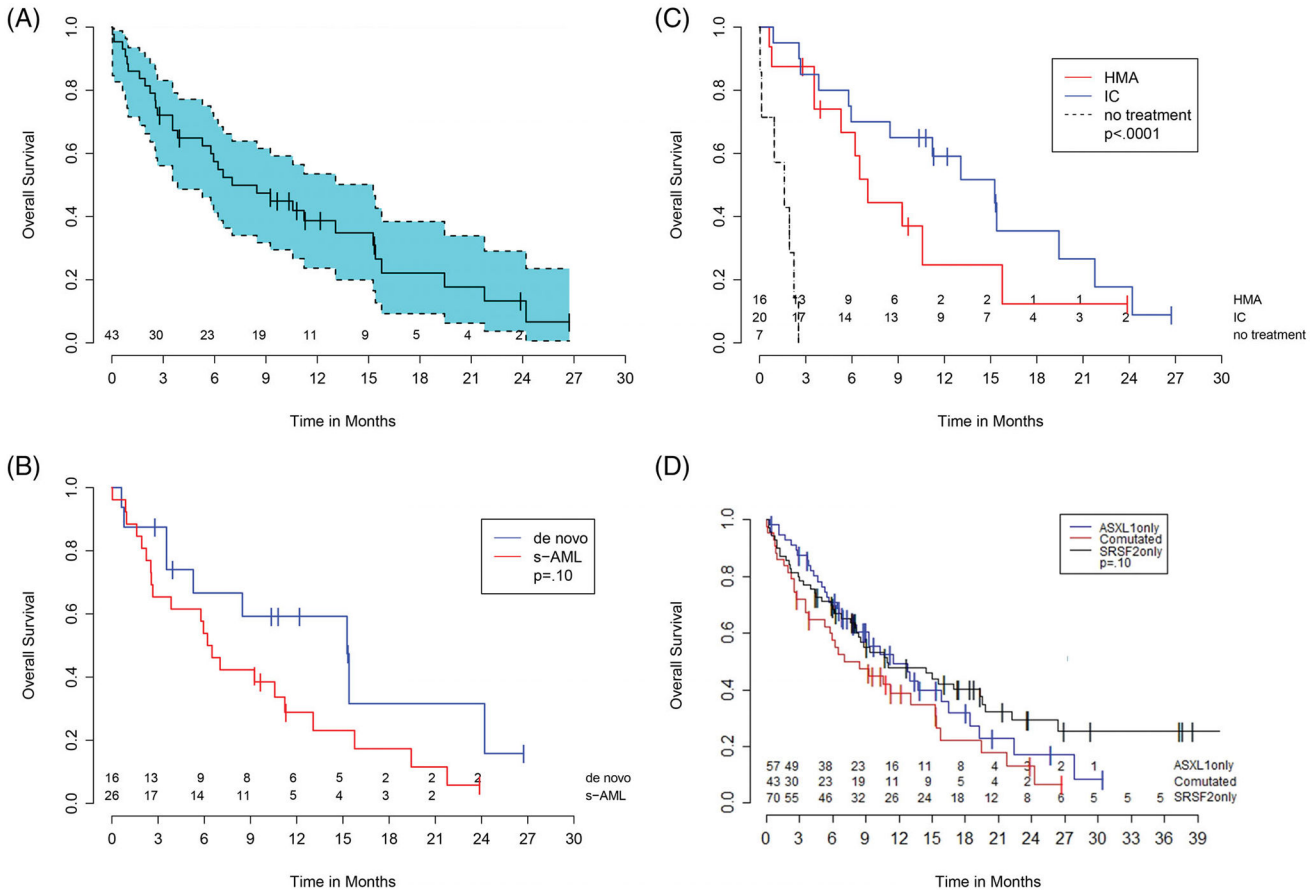

FIGURE 3.

Kaplan–Meier curves of overall survival of *ASXL1*^{mut}*SRSF2*^{mut} AML patients. (A), Entire cohort of *ASXL1*^{mut}*SRSF2*^{mut} AML patients. Blue shading represents the confidence interval. (B), *ASXL1*^{mut}*SRSF2*^{mut} AML cohort stratified by secondary-AML (s-AML) vs de novo AML. (D), *ASXL1*^{mut}*SRSF2*^{mut} AML cohort stratified by treatment intensity (intensive induction chemotherapy [IC] vs hypomethylating agent [HMA] vs no-treatment). (D), *ASXL1*^{mut}*SRSF2*^{mut} AML (comutated) vs *ASXL1*^{mut}*SRSF2*^{wt} AML (*ASXL1* only) vs *ASXL1*^{wt}*SRSF2*^{mut} AML (*SRSF2* only)

TABLE 1

Baseline characteristics of the cohort of patients with *ASXL1*^{mut}*SRSF2*^{mut} AML

	All Patients (n = 43)	De novo AML (n = 16)	Secondary AML (n = 26)	Treatment-related AML (n = 1)	*P-value*
Median Age at Dx—yr. (range)	71 (42–85)	70 (42–83)	72 (59–85)	69	.11
Male sex—no. (%)	30 (70%)	11 (69%)	19 (73%)	1	.43
Race—no. (%)					.72
White	34 (81%)	11 (69%)	22 (85%)	n/a	
African-American	3 (7%)	2 (13%)	1 (4%)		
Hispanic	1 (2%)	0 (0%)	1 (4%)		
Other	4 (9%)	2 (13%)	2 (8%)		
Unknown	1 (2%)	1 (6%)	0 (0%)		
ECOG—no. (%)					.49
0–1	31 (72%)	12 (75%)	19 (73%)		
2–4	10 (23%)	3 (19%)	6 (23%)		
Median WBC at Dx, 10^9/L (range)	8.2 (0.6–157.5)	10.1 (0.9–157.5)	8.0 (0.6–94.3)	86.1	.3
Median Hgb at Dx, g/dL (range)	9.2 (6.1–13.8)	9.9 (6.1–13.8)	9.0 (7.2–13.4)	10.7	.75
Median Plt at Dx, 10^9/L (range)	63 (10–273)	82 (12–199)	57 (10–273)	116	.63
Median LDH at Dx, U/L (range)	406 (166–7960)	958 (166–7960)	370 (171–4435)	3874	.1
Median peripheral blasts % at Dx (range)	7% (0%−80%)	12% (0%−80%)	7% (0%−73%)	35%	.41
Median bone marrow blast % at Dx (range)	36% (0.5%−93%)	64% (24%−93%)	30% (0.5%−85%)	n/a	<.001
Splenomegaly					.07
Present	12 (28%)	3 (20%)	9 (35%)		
Absent	16 (37%)	10 (63%)	6 (23%)		
Total mutations (range)	4 (2–10)	5 (2–7)	4 (2–9)	6	.27
WHO classification					<.001
AML	20 (47%)	13 (81%)	6 (23%)	1	
AML-MRC	23 (53%)	3 (19%)	20 (76%)		
Cytogenetics—no. (%)					.83
ELN Intermediate risk	34 (79%)	11 (67%)	22 (85%)	1	
ELN Adverse risk	6 (14%)	4 (25%)	2 (7%)		
Unknown	3 (7%)	1 (6%)	2 (7%)		

	All Patients (n = 43)	De novo AML (n = 16)	Secondary AML (n = 26)	Treatment-related AML (n = 1)	*P-value*
Induction regimens—no. (%)					.058
Intensive induction	20 (47%)	9 (56%)	11 (42%)		
Hypomethylating agents	16 (37%)	7 (44%)	9 (35%)		
No AML directed chemotherapy	7 (16%)	0 (0%)	6 (23%)	1 (100%)	
Bone marrow transplant no. (%)	6 (14%)	4 (25%)	2 (8%)		.29

Abbreviations: ANC, absolute neutrophil count; Dx, diagnosis; ECOG, Eastern Cooperative Oncology Group; ELN, European Leukemia Network; Hgb, Hemoglobin; LDH, Lactate dehydrogenase; MRC, myelodysplasia-related changes; Plt, Platelet count; WBC, white blood cell count;