

## TO THE EDITOR:

## Clinical, molecular, and prognostic comparisons between CCUS and lower-risk MDS: a study of 187 molecularly annotated patients

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Clonal cytopenia of undetermined significance (CCUS) is defined by the presence of somatic driver mutations/copy number alterations (CNA) in hematopoietic cells, in patients with unexplained cytopenia in 1 or more peripheral blood (PB)-cell lineages, in the absence of overt morphologic dysplasia, excess blasts, and myelodysplastic syndromes (MDS)-defining chromosomal abnormalities.<sup>1,2</sup> CCUS is associated with an increased risk for evolution to MDS and/or acute myeloid leukemia (AML), with somatic mutational variant allele frequency (VAF)  $\geq 10\%$ , or carrying  $\geq 2$  mutations, being associated with predictive values of 0.86 and 0.88 for diagnosis of a myeloid neoplasm.<sup>1</sup> In addition, dominant clone VAF  $> 20\%$  has been associated with a  $> 95\%$  risk of progression to a clinically apparent myeloid neoplasm in 10 years, raising the question as to whether these patients would better be classified as having an early myeloid neoplasm rather than CCUS.<sup>3</sup> These data, among others, have led to operational classification criteria for the diagnosis of CCUS that includes the presence of myeloid-relevant somatic mutations with a VAF  $\geq 20\%$ , without overt bone marrow (BM) dysplasia ( $< 10\%$  dysplastic cells).<sup>2</sup>

MDS are clonal hematopoietic stem cell disorders with clinically relevant morphologic dysplasia in the PB and BM, with  $< 20\%$  blasts and an inherent risk for AML transformation.<sup>4</sup> Lower-risk MDS (LR) can be further categorized into morphologic and molecularly annotated entities that have  $< 5\%$  blasts, with lower rates of AML progression but with significant quality of life issues and red blood cell and platelet transfusion dependence (TD).<sup>2,4</sup> With the advent of clinical next-generation sequencing (NGS) testing, there are increasing numbers of patients with unexplained cytopenias not meeting criteria for MDS that have somatic mutations/CNA suggestive of CCUS.<sup>5</sup> Although these patients are currently not considered to have a hematologic neoplasm, they can be as symptomatic, if not more, in comparison with patients with MDS. However, because of the lack of a formal cancer diagnosis, CCUS patients are often denied MDS-directed therapies or clinical trial enrollment. We carried out this study to compare and contrast the clinical characteristics and survival outcomes between prospectively assessed patients with CCUS at our institution and LR-MDS patients.

CCUS patients were identified from the Mayo Clinic clonal hematopoiesis (CH) clinic, whereas LR-MDS patients were retrospectively identified from our institutional database. For this study, we defined LR-MDS by the 2016 World Health Organization (WHO) criteria for MDS, as long as they were stratified as having very-low-, low-, and intermediate-risk disease by the revised International Prognostic Scoring System (R-IPSS).<sup>6</sup> The gold standard criteria applied for dysplasia included the presence of  $\geq 10\%$  dysplastic erythroid and/or granulocytic cells and  $\geq 30\%$  dysplastic megakaryocytes, with micro-megakaryocytes and multinucleated megakaryocytes with separated nuclei being the most reliable dysplastic finding in the megakaryocyte lineage.<sup>4</sup> Depending on the extent of dysplasia, further subcategorization into single lineage dysplasia (SLD) and multilineage dysplasia (MLD) was carried out. Although consensus data have suggested using VAF  $\geq 20\%$  for the diagnosis of CCUS, in our study, we included patients with a VAF  $\geq 2\%$ , based on CH criteria and the fact that formal validation of CCUS

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All raw sequencing data will be made available on e-mail request to the corresponding author at patnaik.mrinal@mayo.edu. Individual variant details are provided in the supplemental files.

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**Table 1. Clinical characteristics of CCUS and LR-MDS**

Variables	All patients with CCUS and LR-MDS (n = 187)	CCUS (n = 75)	LR-MDS (n = 112)	P
<b>Demographics, median (range)</b>				
Age, y	66 (20-91)	65 (20-90)	67 (37-91)	.796
Sex; males, n (%)	118 (63.1)	51 (68)	67 (59.8)	.282
<b>Blood counts at diagnosis, median (range)</b>				
HGB, g/dL	10.6 (5.8-15.9)	10.8 (6.7-15.9)	10.4 (5.8-14.4)	.307
MCV, fL	99.7 (80.4-120.2)	99.6 (80.4-117.2)	99.6 (81.5-120.2)	.850
WBC, $\times 10^9/L$	4.7 (0.9-13.1)	4.1 (1.2-13.1)	5.1 (0.9-13.1)	<b>.002</b>
ANC, $\times 10^9/L$	2.5 (0.04-9.4)	2.1 (0.04-9.4)	2.7 (0.2-9.4)	<b>.008</b>
AMC, $\times 10^9/L$	0.4 (0-3.6)	0.5 (0.01-3.6)	0.3 (0-1.1)	<b>.007</b>
PLT, $\times 10^9/L$	196 (12-599)	140 (10-595)	232 (12-599)	<b>&lt;.001</b>
Myelocytes, %	0.9 (0-24)	1.1 (0-24)	0.7 (0-19)	.324
Metamyelocytes, %	0.5 (0-11)	0.5 (0-11)	0.4 (0-10)	.647
Peripheral blasts, %	0.04 (0-2)	0.03 (0-1)	0.04 (0-2)	.796
<b>Transfusion dependency, n (%)*</b>				
Red blood cell	66 (35.3)	17 (22.6)	49 (43.7)	<b>.003</b>
Low burden	32 (48.5)	9 (53)	23 (46.9)	
High burden	35 (53)	9 (53)	26 (53.1)	<b>.025</b>
Platelets	24 (12.8)	9 (12)	15 (13.4)	.827
<b>Bone marrow characteristics, n (%)</b>				
Hypercellular	132 (71)	43 (57.3)	89 (83.2)	<b>.001</b>
Hypocellular	14 (7.8)	5 (6.9)	9 (8.4)	.784
Normocellular	33 (18.3)	24 (33.3)	9 (8.4)	<b>&lt;.001</b>
Abnormal MDS flow cytometry	22 (11.7)	12 (16)	10 (8.9)	<b>&lt;.001</b>
BM ring sideroblasts, median (range), %	16 (0-75)	2 (0-5)	24 (0-75)	<b>&lt;.001</b>
BM blasts, median (range), %	1.1 (0-15)	0.7 (0-5)	1.4 (0-15)	<b>.002</b>
Abnormal cytogenetics	44 (23.5)	16 (21.3)	28 (25)	.602
Mutation present on NGS	163 (87.2)	67 (89.3)	96 (85.7)	.251
1 mutation	61 (37.4)	22 (32.8)	39 (40.6)	.329
>1 mutation	102 (62.6)	45 (67.2)	57 (59.4)	
<b>IPSS-R cytogenetic risk category, n (%)</b>				
Very good	8 (4.3)	2 (2.7)	6 (5.4)	.483
Good	161 (87)	63 (86.3)	98 (87.5)	.826
Intermediate	12 (6.5)	5 (6.8)	7 (6.3)	.98
Poor	4 (2.2)	3 (4.1)	1 (0.9)	.302
Very poor	0 (0)	0 (0)	0 (0)	—
<b>NGS analysis, n (%)</b>				
Epigenetic regulators				
<i>DNMT3A</i>	29 (17.4)	9 (12.7)	20 (20.8)	.216
<i>IDH1</i>	6 (3.6)	6 (8.5)	0 (0)	<b>.005</b>
<i>IDH2</i>	5 (3)	2 (2.8)	3 (3.1)	.65
<i>KDM6A</i>	1 (0.6)	1 (1.4)	0 (0)	.420
<i>TET2</i>	49 (29.2)	21 (29.6)	28 (28.6)	.87
Chromatin regulators				
<i>ASXL1</i>	23 (13.7)	9 (12.7)	14 (14.6)	.822
<i>EZH2</i>	1 (0.6)	0 (0)	1 (1)	.88

HGB, hemoglobin; IPSS-R, International prognostic scoring system revised; MCV, mean corpuscular volume; PLT, platelets; —, lower-risk myelodysplastic syndromes.

\*Transfusion dependency defined by requirement of at least 1 unit of red cell or platelet transfusion once every 4 weeks for more than 2 consecutive 4-week periods. Low burden defined as 3 to 7 units in a 16-week period; high burden defined as 8+ units in a 16-week period.<sup>12</sup>

†Although IPSS-R was designed for MDS, we also applied it to CCUS patients.

#Median OS reached for MDS at 104 months was not reached for CCUS..

**Table 1. (continued)**

Variables	All patients with CCUS and LR-MDS (n = 187)	CCUS (n = 75)	LR-MDS (n = 112)	P
<b>Spliceosome factors</b>				
<i>SRSF2</i>	25 (14.7)	14 (20)	11 (11.2)	.132
<i>SF3B1</i>	70 (41.2)	4 (5.6)	66 (67.4)	<.001
<i>U2AF1</i>	14 (8.2)	8 (11.1)	6 (6.1)	.266
<i>ZRSR2</i>	11 (6.5)	10 (14.1)	1 (1)	.001
<b>Cell signaling</b>				
<i>CBL</i>	5 (3)	1 (1.4)	4 (4.2)	.396
<i>CSF3R</i>	8 (4.8)	2 (2.8)	6 (6.3)	.469
<i>JAK2</i>	5 (3)	3 (4.2)	2 (2)	.652
<i>KIT</i>	1 (0.6)	1 (1.4)	0 (0)	.420
<i>KRAS</i>	3 (1.8)	2 (2.8)	1 (1)	.575
<i>MPL</i>	2 (1.2)	1 (1.4)	1 (1)	.46
<i>NRAS</i>	2 (1.2)	2 (2.8)	0 (0)	.175
<i>SH2B3</i>	1 (0.6)	1 (1.4)	0 (0)	.420
<i>WT1</i>	1 (0.6)	1 (1.4)	0 (0)	.420
<b>Tumor suppressors</b>				
<i>ATM</i>	5 (3)	5 (7)	0 (0)	.013
<i>PTEN</i>	1 (0.6)	1 (1.4)	0 (0)	.418
<i>TP53</i>	6 (3.5)	4 (5.6)	2 (2)	.240
<b>Others</b>				
<i>BCOR</i>	5 (3)	4 (5.6)	1 (1)	.164
<i>CEBPA</i>	5 (3)	0 (0)	5 (5.2)	.073
<i>CHEK2</i>	1 (0.6)	1 (1.4)	0 (0)	.420
<i>ETV6</i>	1 (0.6)	0 (0)	1 (1)	.912
<i>ETNK1</i>	1 (0.6)	0 (0)	1 (1)	.987
<i>PHF6</i>	2 (1.2)	2 (2.8)	0 (0)	.175
<i>RAD21</i>	1 (0.6)	1 (1.4)	0 (0)	.420
<i>RUNX1</i>	2 (1.2)	1 (1.4)	1 (1)	.654
<i>SETBP1</i>	5 (3)	1 (1.4)	4 (4)	.400
<i>STAG2</i>	3 (1.8)	3 (4.2)	0 (0)	.072
<b>IPSS-R risk category; n (%)†</b>				
Very low	81 (43.3)	32 (42.7)	49 (43.8)	.879
Low	80 (42.7)	33 (44)	47 (42)	.880
Intermediate	26 (13.9)	10 (13.3)	16 (14.3)	.986
High	0 (0)	0 (0)	0 (0)	—
Very high	0 (0)	0 (0)	0 (0)	—
<b>Treatment type, n (%)</b>				
None	87 (47.5)	58 (77.3)	29 (26.9)	<.001
Erythropoietin stimulators	47 (25.7)	8 (10.7)	39 (34.8)	<.001
Immune suppression	13 (7.1)	2 (2.6)	11 (10.2)	.077
Hypomethylating agent	27 (14.8)	7 (9.3)	20 (18.5)	.094
Bone marrow transplant	9 (4.9)	0	9 (8.3)	.011
<b>Clinical course</b>				
Disease progression, n (%)	22 (11.8)	13 (17.3)	9 (8)	.067
To MDS/chronic myeloid neoplasm	11 (50)	10 (76.9)	1 (11.1)	.008

HGB, hemoglobin; IPSS-R, International prognostic scoring system revised; MCV, mean corpuscular volume; PLT, platelets; —, lower-risk myelodysplastic syndromes.

\*Transfusion dependency defined by requirement of at least 1 unit of red cell or platelet transfusion once every 4 weeks for more than 2 consecutive 4-week periods. Low burden defined as 3 to 7 units in a 16-week period; high burden defined as 8+ units in a 16-week period.<sup>12</sup>

†Although IPSS-R was designed for MDS, we also applied it to CCUS patients.

‡Median OS reached for MDS at 104 months was not reached for CCUS.

**Table 1. (continued)**

Variables	All patients with CCUS and LR-MDS (n = 187)	CCUS (n = 75)	LR-MDS (n = 112)	P
To AML	11 (5.0)	3 (23.1)	8 (88.9)	
Alive, n (%)	120 (64.2)	60 (80)	60 (53.6)	<.001
Follow-up, median (range, mo)	32 (0.1-238)	17 (2-125)	54 (0.1-238)‡	<.001

HGB, hemoglobin; IPSS-R, International prognostic scoring system revised; MCV, mean corpuscular volume; PLT, platelets; —, lower-risk myelodysplastic syndromes.

\*Transfusion dependency defined by requirement of at least 1 unit of red cell or platelet transfusion once every 4 weeks for more than 2 consecutive 4-week periods. Low burden defined as 3 to 7 units in a 16-week period; high burden defined as 8+ units in a 16-week period.<sup>12</sup>

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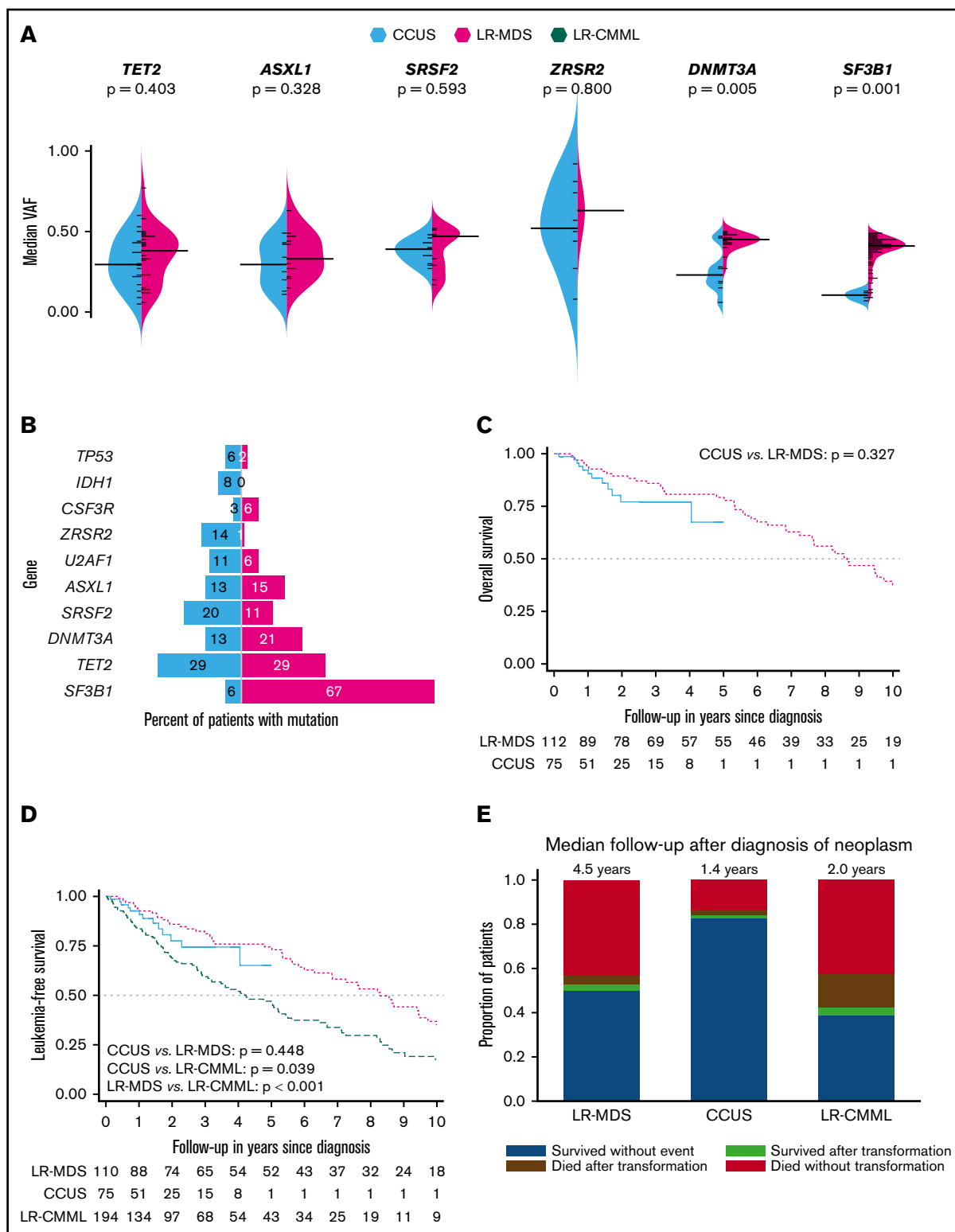
consensus criteria are pending.<sup>7,8</sup> In our study we further classified CCUS patients into CCUS-HighVAF based on a dominant mutational VAF of  $\geq 20\%$ . In addition, patients with unexplained cytopenias with somatic copy number alterations (SCNA), excluding MDS-defining cytogenetic abnormalities, were also classified as having CCUS.<sup>9</sup> Baseline demographics, blood counts, BM morphology, cytogenetics, and NGS results (supplemental Table 1 for NGS panel details) were abstracted. Although the IPSS and R-IPSS prognostication systems have been developed and validated strictly for newly diagnosed MDS patients, given the clinical similarity and lack of a CCUS-specific risk stratification system, in this study, we applied the R-IPSS risk stratification to CCUS patients. Transfusion dependency was defined as requiring at least 1 unit of red cell or platelets every 4 weeks for 2 or more consecutive months. The Mann-Whitney *U* and Fischer's exact test were used to compare quantitative and qualitative data in subgroups. Kaplan-Meier overall survival (OS) estimates were used for survival analysis and compared using the log-rank test.

A total of 187 patients were included in the study: 75 (40%) with CCUS and 112 (60%) with LR-MDS, with a median age of 66 years (63% male; Table 1). In the CCUS group, 59 (78%) patients had  $\geq 1$  somatic mutation, 8 (11%) had clonal cytogenetic abnormalities only, and 8 (11%) patients had both somatic mutations and clonal cytogenetic abnormalities. Sixty-seven (92%) patients had  $\geq 1$  somatic mutation, with 45 (61%) having  $>1$  mutation. Common mutations in CCUS included *TET2* (30%), *SRSF2* (20%), *DNMT3A* (13%), and *ASXL1* (11%) (Figure 1A-B), with 17 (23%) patients being red cell TD and 10 (13%) being platelet TD at diagnosis. Of note, the higher-than-expected TD rates in the CCUS cohort could be secondary to the fact that our institution is a tertiary referral center and that the CH clinic is a specialty referral clinic, introducing an element of referral bias. Based on the R-IPSS stratification system for MDS, 32 (42%), 33 (43%), and 10 (13%) CCUS patients were stratified as having very-low-, low-, and intermediate-risk disease, respectively. In the LR-MDS group, MDS subtypes as defined by the 2016 WHO criteria included 56 (50%) patients with MDS-ring sideroblasts (RS)-SLD, 4 (4%) MDS-SLD, 23 (21%) MDS-MLD, 11 (10%) MDS-EB-1 (excess blasts), 8 (7%) MDS with del(5q), and 10 (9%) MDS-unclassifiable. Ninety-six (86%) LR-MDS patients had  $\geq 1$  somatic mutation, whereas 57 (51%) had  $>1$  mutation, with common mutations being *SF3B1* 67% (95% MDS-RS), *TET2* (29%), and *DNMT3A* (21%). For LR-MDS patients, 49 (44%) were red cell TD, whereas 15 (13%) were platelet TD. On R-IPSS stratification, 49 (44%), 47 (42%), and 16 (14%) MDS patients were stratified as having very-low-, low-, and intermediate-risk disease, respectively.

In comparison with patients with CCUS, LR-MDS patients were more likely to have higher white blood cell counts (WBC;  $P = .001$ ), higher absolute neutrophil counts (ANC;  $P = .005$ ), higher platelet counts ( $P < .001$ ), be red cell TD ( $P = .031$ ), have BM RS ( $P < .001$ ), have higher BM blast% ( $P = .002$ ), and have *SF3B1* mutations ( $P < .001$ ), whereas CCUS patients were more likely to have higher absolute monocyte counts (AMC;  $P = .009$ ; Table 1). Mutational VAF for *DNMT3A* and *SF3B1* was higher in LR-MDS in comparison with CCUS (Figure 1A). Importantly, there were no differences with regard to R-IPSS cytogenetic risk groups, R-IPSS prognostic categories, platelet TD, and the distribution of *TET2*, *DNMT3A*, *SRSF2*, *ASXL1*, and *TP53* mutations.

Fifty (83%) of 60 assessable CCUS patients with somatic mutations had at least 1 somatic mutation with VAF  $\geq 20\%$  (CCUS-HighVAF), whereas 58 (97%) had VAF  $\geq 10\%$  (supplemental Table 2). In comparison with CCUS patients with VAF  $< 20\%$ , CCUS-highVAF patients were more likely to have higher AMC ( $P = .007$ ), less likely to have hypocellular BM ( $P = .015$ ), more likely to have  $>1$  somatic mutation (76% vs 20%,  $P = .001$ ), more likely to have *TET2* mutations ( $P = .025$ ), and less likely to have *TP53* mutations ( $P = .013$ ; supplemental Table 3). When CCUS-highVAF patients were compared with LR-MDS patients, they were more likely to have higher AMC ( $P < .001$ ), lower platelet counts ( $P < .001$ ), less likely to be RBC TD ( $P = .036$ ), less likely to have BM RS ( $P < .001$ ), have lower BM blasts% ( $P = .001$ ), more likely to have  $>1$  mutation ( $P = .001$ ), and less likely to have *SF3B1* mutations ( $P < .001$ ) (supplemental Table 4). We also compared the CCUS cohort to 239 WHO-defined lower risk patients with chronic myelomonocytic leukemia (CMML), an MDS/myeloproliferative overlap neoplasm, with risk stratification based on the Mayo Molecular Model.<sup>10</sup> In comparison with LR-CMML, CCUS patients were younger in age ( $P = .003$ ), more anemic ( $P = .001$ ) and thrombocytopenic ( $P = .023$ ), had lower WBC, ANC, and AMC ( $P < .001$  for each), had lower PB blasts % ( $P = .023$ ), and were less likely to have mutations involving *TET2* ( $P = .021$ ), *SRSF2* ( $P < .001$ ), *ASXL1* ( $P = .027$ ), *CBL* ( $P = .001$ ), and *SETBP1* ( $P = .010$ ; supplemental Table 5). Of note, CMML arises in the context of age-related clonal hematopoiesis with *TET2/SRSF2* mutations skewing hematopoiesis with a monocyte bias and is relatively enriched in *ASXL1* (40%), *CBL* (15%), and *SETBP1* (15%) mutations.<sup>11</sup>

LR-MDS patients had a longer median follow-up (54 vs 16 months), and at last follow-up, 52 (54%) and 12 (16%) deaths have been documented in the LR-MDS and CCUS groups, respectively (5 of 50 in CCUS-HighVAF). Of note, the longer follow-up of the LR-MDS patients in comparison with CCUS patients was in part because of the recent establishment of the CH clinic at our institute



**Figure 1. Molecular characteristics and survival outcomes in patients with clonal cytopenias of undetermined significance, lower-risk myelodysplastic syndromes, and lower-risk chronic myelomonocytic leukemia.** (A) Split violin plot demonstrating the 6 most common mutations in both CCUS and LR-MDS and their respective VAFs. (B) Bar graph of CCUS and LR-MDS demonstrating the 10 most frequent mutations in all 188 patients and the percentage of patients with that mutation. (C) Kaplan-Meier OS estimate in CCUS and LR-MDS patients, censored at 10 years of follow-up. (D) Kaplan-Meier leukemia-free survival estimate in CCUS, LR-MDS, and LR-CMML patients, censored at 10 years of follow-up. (E) Distribution of survival and progression outcomes in LR-MDS, LR-CMML, and CCUS patients.



(2017) and in part because of the recent creation of the CCUS nosologic category.<sup>1,3</sup> Regardless, ongoing follow-up of the CCUS cohort will be very valuable in terms of longer-term outcomes including clonal evolution and OS. CCUS and LR-MDS patients were followed clinically, and BM biopsies were carried out based on clinical need and suspicion to assess for disease progression (no routine/serial biopsies were done in either cohort). Thirteen (18%) CCUS patients progressed to MDS/CMML ( $n = 10$ , MDS-8, CMML-2; 6 with CCUS-HighVAF) and AML/blastic plasmacytoid dendritic cell neoplasm ( $n = 3$ , AML-2, BPDCN-1; 2 with CCUS-HighVAF) over a median of 16 months, whereas 9 (8%) LR-MDS patients progressed to higher-grade MDS (1) and AML (8), respectively.

To further delineate predictors of clonal evolution, CCUS patients were analyzed using a univariate logistic regression for the binary outcome of progression to myeloid neoplasm vs nonprogression. Once again, within limitation of a small sample size, several variables (supplemental Table 6) were tested, with higher risk cytogenetic groups by R-IPSS ( $P = .018$ ) and the presence of *SF3B1* mutations ( $P = .014$ ) being statistically associated with clonal evolution/disease progression. We also reviewed the individual prognostic variables contributing to the R-IPSS scores in CCUS and LR-MDS patients (supplemental Table 7) and found that in CCUS patients, 80 (84%) of a cumulative 95 prognostic points were contributed by cytopenias (38% from anemia), whereas in the LR-MDS group, 84 (76%) of a cumulative 110 prognostic points were contributed by cytopenias (55% from anemia). This is explained by the fact that in both categories, karyotypic abnormalities and increased BM blasts were infrequent.

Although hypomethylating agents (HMAs) have not been approved for the management of CCUS, 7 (9%) CCUS patients and 20 (18%) LR-MDS patients treated with HMAs were assessable for response, with 1 patient each in the CCUS group meeting the proposed 2018 International Working Group MDS response criteria for hematologic improvement (HI)–erythroid, HI-platelet, and HI-neutrophil, respectively, in comparison with 4 (20%), 2 (10%), and 1 (5%) patients in the LR-MDS group.<sup>12</sup> Three of 8 (37%) CCUS patients and 13 (33%) of 39 LR-MDS patients treated with erythropoiesis-stimulating agents (ESA) met criteria for HI-erythroid.

There was no difference in median OS between CCUS, including CCUS-HighVAF (median OS not reached) vs LR-MDS (median OS, 8.3 years) patients ( $P = .337$ ; Figure 1C). Within limitations of a shorter follow-up, there was no OS difference between CCUS-HighVAF and CCUS-LowVAF ( $P = .524$ ) patients or among CCUS patients further stratified by the number of mutations/SCNA ( $P = .288$ ). Within limitations of a small sample size, we estimated the competing risk for AML progression on all 3 cohorts of patients using the Fine and Gray competing risk model.<sup>13</sup> LR-CMML patients were less likely to transform to AML in comparison with LR-MDS patients, whereas the risk in CCUS patients was not significantly different from LR-MDS and LR-CCUS patients (Figure 1D-E).

Increased use of NGS testing has resulted in a higher prevalence of CCUS.<sup>5</sup> Because of the lack of a formal neoplastic diagnosis, these patients are often managed with supportive care and are not eligible for MDS-approved therapies and clinical trials. We demonstrate

that apart from subtle phenotypic and molecular differences, CCUS patients have overlapping similarities with LR-MDS, including the distribution of common MDS-driver mutations (*DNMT3A*, *TET2*, and *ASXL1*), with the exception of mutant *SF3B1*, which associates strongly with a diagnosis of MDS-RS.<sup>14,15</sup> Although LR-MDS patients were more likely to be red cell TD (44%), 24% of CCUS patients were also red cell TD, with 13% in both groups being platelet TD. We observed higher WBC/ANC and platelet counts in MDS patients in comparison with CCUS patients, largely because of the fact that 50% of the MDS cohort comprised of patients with MDS-RS-SLD, where the dysplasia is largely limited to the erythroid lineage, sparing granulocyte and megakaryocyte development and maturation, and is a limitation of our study. Importantly, although the R-IPSS is currently applicable to MDS patients only, when applied to CCUS patients, risk stratification was similar. In addition, within a short follow-up period, 17% of CCUS patients progressed to MDS/AML, exemplifying that these entities exist in the same spectrum of myeloid neoplasms. Eighty-three percent of CCUS patients had a VAF  $\geq 20\%$ , whereas 91% had a VAF  $\geq 10\%$ , providing credibility to using VAF  $\geq 10\%$  for a diagnosis of CCUS, with further prospective validation needed.

This study is limited by the length of follow up for CCUS (16 months) compared with LR-MDS patients (54 months) and the fact that there could be a selection bias in detection of CCUS patients who have significant cytopenias and comorbidities, because they are more likely to seek care. In addition, given that 50% of our LR-MDS patients had MDS-RS-SLD, where *SF3B1* is a dominant mutation and where BM dysplasia is largely restricted to the erythroid lineage, this could certainly influence phenotypic/molecular comparisons between LR-MDS and CCUS patients. However, despite these limitations, we demonstrate the evident clinical and molecular overlap between CCUS and LR-MDS patients and strongly urge the academic community to consider including CCUS, especially CCUS-HighVAF ( $\geq 20\%$ ), as a MDS subtype in the next iteration of the WHO classification system. In addition, given the lack of a prognostication system, we also urge the MDS consortium to consider assessing CCUS-HighVAF patients as they develop the IPSS-Molecular model. These steps will ensure better care for CCUS patients and importantly provide access to enrollment in MDS-directed clinical trials.

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