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Characteristics of clonal cytopenia of undetermined significance in the presence of *ZRSR2* mutations

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Running title: *ZRSR2*-mutated CCUS

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To The Editor,

Clonal cytopenia of undetermined significance (CCUS) is defined by clonal hematopoiesis with myeloid malignancy–associated mutations (variant allele frequency ≥2%) and one or more unexplained persistent cytopenias that do not meet criteria for a defined myeloid neoplasm (MN).[1] CCUS is considered a precursor of MN.[2, 3] *ZRSR2* (x-linked) is a myeloid neoplasm associated gene and a member of the spliceosome family of genes, which also includes *SF3B1*, *SRSF2*, and *U2AF1.[4-7]* Both CCUS and *ZRSR2* remain poorly understood, and their rarity has limited previous studies.[8] However, we and others found CCUS patients to be enriched in *ZRSR2* mutations compared to those with MN.[9, 10] This study aimed to assess the impact of *ZRSR2* mutations on CCUS progression and survival.

Next-generation sequencing (NGS) was performed on 9,320 patients' samples collected between 2016-2024 at Mayo Clinic.[11] This study was approved by the Mayo Clinic IRB (#15-004211) and conducted in accordance with the Declaration of Helsinki. Given the retrospective nature of the study, informed consent was waived. Clinical characteristics, bone marrow morphology, cytogenetics, and molecular study results were abstracted. Overall survival (OS) was measured using Kaplan-Meier estimates from the date of NGS to death or last follow-up comparing with different cohorts (MN CCUS patients). BlueSky Statistics V10.3.1 was used for data analysis.

A total of 40 patients were identified harboring both CCUS and *ZRSR2* mutations (*ZRSR2m*). All patients were male. Only 4 patients were diagnosed prior to NGS date, 2 with CCUS (6 years prior) and 2 with previously treated AML (1 and 13 years prior). Eight patients (20%) had concurrent hematological malignancies, including 5 (12.5%) with plasma cell proliferative disorders (3 multiple myeloma (MM) and 1 monoclonal gammopathy of undetermined significance (MGUS)). Other malignancies included 2 chronic lymphocytic leukemias (CLL) and 1 with VEXAS syndrome.

Five patients (12.5%) received prior chemotherapy, with one also receiving radiotherapy. Thirteen of the 40 patients (32.5%) received non-chemotherapy prior to diagnosis including rituximab, tocilizumab, acalabrutinib, mycophenolate mofetil, methotrexate (2 patients at low doses for autoimmune/rheumatological conditions), or a proteasome inhibitor like carfilzomib for a variety of neoplastic conditions (T-cell large granular lymphocytic leukemia, CLL, follicular lymphoma, MM, VEXAS, plasma cell proliferative disorder/primary amyloidosis, and non-neoplastic disorders like inflammatory arthritis). (Table 1)

Abnormal cytogenetics were present in 11 patients (27.5%), with +8 being the most common (n=5, 12.8%). Other abnormalities included -Y, del(20), del(9), t(1;3), -13, and +mar.

ZRSR2m median gender-corrected variant allele frequency (VAF) among CCUS patients was 31% (mean, 28%, range, 1-44.5). Six patients (15%) had isolated *ZRSR2m*, and 1 (2.5%) had multiple *ZRSR2m*, while the median number of co-mutations was 1 (range, 0-4). Sequential NGS showed 1 patient developed 3 *ZRSR2* mutations and 2 *ZRSR2* variants of unknown significance. *TET2m* was the most common co-mutation present in 25 (62.5%) of the CCUS patients, *TET2m* was the isolated co-mutation in 18 out of the 33 patients (54.5%) who had mutations other than *ZRSR2m*. *ASXL1* mutation was the second most common (n= 8, 20%), other less common co-mutations were *IDH1*, *IDH2*, *SRSF2*, *SF3B1*, *TP53*, *GATA2*, *KDM6A*, *PPM1D*, and *UBA1*. (Supplemental Figure 1)

Pre-ZF1 was the most common domain for *ZRSR2* mutations (n= 23, 57.5%) followed by post-ZF2 (n=7, 17.5%). Other sites for *ZRSR2* mutations were in ZF1, ZF2 and UHM domains. Mutations were exclusively nonsense (40%), frameshift (37.5%), and splice site (22.5%).

According to the clonal hematopoiesis risk score (CHRS), 37 patients (92.5%) were classified as high risk and 3 (7.5%) as intermediate risk. Although most patients were classified as high risk, overall survival did not differ significantly according to CHRS risk category (Supplementary Figure 2). With a median follow-up of 30 months, none of the intermediate risk *ZRSR2m* CCUS patients progressed to MN and 9 (24.3%) of high-risk patients progressed to MN including 5 (13.5%) patients progressing to MDS, 4 of them subclassified as low blast (LB) and 1 as increased blast-1 (IB1) with median time to MDS progression being 13 months. Four (10.8%) progressed to MDS/MPN overlap (3 CMML). However, 24 of the 40 (60%) CCUS patients showed absolute monocyte count of ≥0.5 x 10^9/L indicating a monocytic lineage preference (known as clonal cytopenia and monocytosis of undetermined significance (CCMUS)). None of the patients who had isolated *ZRSR2m* progressed to either MDS or CMML. None of the patients who received prior immunotherapy progressed to MDS (p=0.09). (Table 1)

Twelve (30%) patients died, with a median overall survival (mOS) of 56 months. (Figure 1, A) Patients with isolated *TET2*m comutation showed better mOS than the rest of the cohort (not reached vs 41 months, p= 0.025). (Figure 1, B) Improved OS was noted among patients with higher hemoglobin concentrations (HR=0.59, p=0.016) and lower *ZRSR2* VAF (HR=1.05, p=0.016). *ZRSR2m* CCUS patients showed better mOS than *ZRSR2m* with MNs but did not reach statistical significance (125 MN patients vs 40 CCUS patients, 34 vs 56 months, p=0.19). On multivariate analysis, both Hgb concentration (HR=0.61, p=0.03) and isolated *TET2* co-mutation (HR=0.19, p=0.04) retained statistical significance. mOS was not affected by number of co-mutations, isolated *ZRSR2*, *ASXL1* co-mutation, MDS progression or CHRS risk.

Eleven (27.5%) of the 40 patients received therapy (for symptomatic cytopenia) while being diagnosed as CCUS, including steroids, ascorbic acid, azacitidine, darbepoetin alfa, and G-CSF with one patient receiving oral decitabine-cedazuridine. One (3.4%) of 29 untreated patients progressed to MDS, compared to 4 (36.4%) of 11 treated patients (p=0.005). (Figure 1, C) mOS was unaffected by treatment. Thirteen patients (32.5%) had sequential NGS, with 5 gaining additional mutations, including *ZRSR2*, *CBL, UBA1, RUNX1, and ASXL1 mutations*.

Our cohort was predominantly male, consistent with prior reports showing higher *ZRSR2* mutation frequency compared with other X-linked genes. Further studies are needed to understand the underlying mechanism. A substantial proportion of patients (42.5%) had concurrent hematologic malignancies or prior therapies, exceeding the 10–20% typically reported. In therapy-related CCUS (t-CCUS), particularly in patients with prior therapies or concurrent malignancies, distinguishing CCUS from clonal hematopoiesis can be challenging; however, the high VAF in our cohort likely supports a true clonal process.

Despite high-risk classification, CCUS patients showed low progression to myeloid neoplasms, particularly those with isolated *ZRSR2* mutations, suggesting CHRS may underperform in this group and that isolated *ZRSR2* follows an indolent course, unlike other spliceosome mutated CCUS. Patients receiving treatment or classified as high-risk *ZRSR2m* CCUS had higher rates of MDS progression despite low overall risk.

Interestingly, the presence of an isolated *TET2* co-mutation in *ZRSR2m* patients was associated with better survival. This observation suggests that *TET2* mutations, when occurring alongside *ZRSR2*, may exert a more indolent disease course in CCUS patients. This finding was reported in patients with myeloid neoplasms carrying these two concurrent mutations. Monocytosis was assessed in this cohort, and its association with *ZRSR2* has been previously described[10, 12, 13]

Our study was limited by retrospective nature, the use of in-house NGS with limited panels, small sample size for a rarely mutated gene, heterogeneity of prior treatments, and potential other confounding factors.

In summary, while the *ZRSR2* mutation is commonly observed in male patients with CCUS, the clinical course of these patients appears to be less aggressive than anticipated. The presence of *TET2* as a co-mutation seems to further improve the prognosis, indicating that genetic profiling plays a crucial role in determining the clinical outcomes for CCUS patients with *ZRSR2* mutations. Classic prognostic models for CCUS progression prediction may not perform well in this unique group. Future studies should focus on molecular mechanisms underlying these observations to guide more tailored management strategies.

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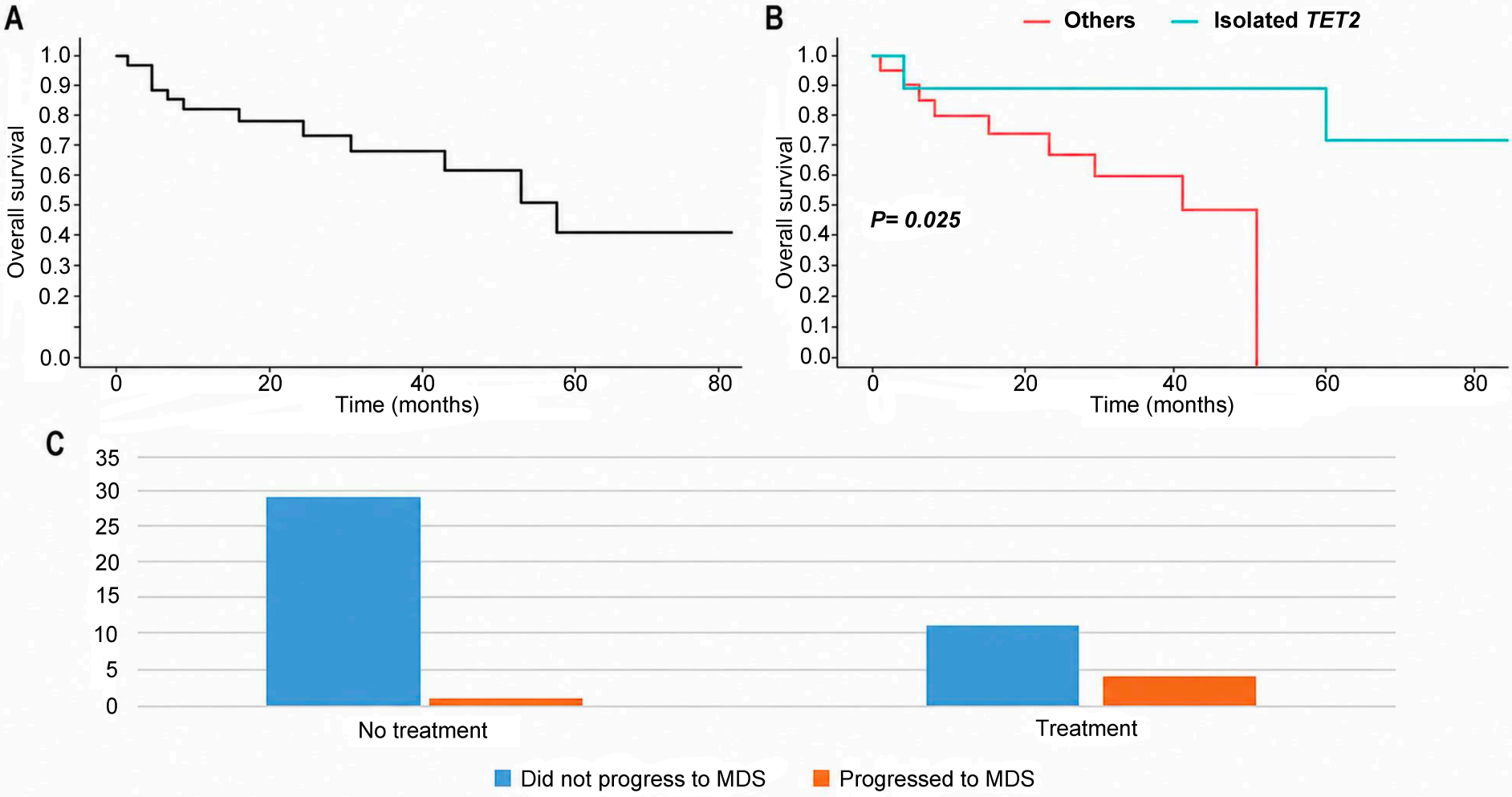
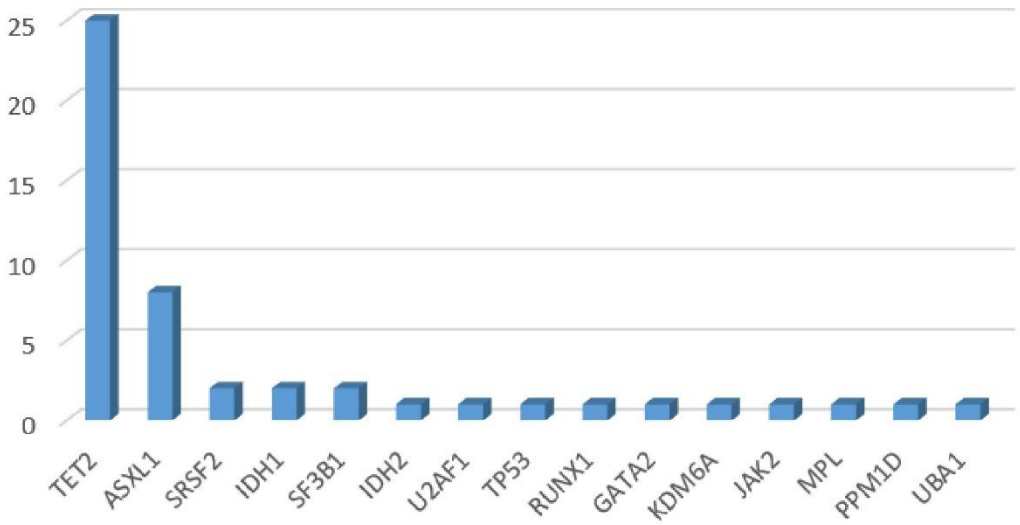
Table 1. Patients' characteristics for *ZRSR2m* CCUS patients

Number of Patients	**40**
Male sex, n (%)	40 (100.0%)
Age, Median (q1, q3) years	76 (72.5, 78)
Prior chemotherapy or radiotherapy, n (%)	5 (12.5%)
Prior Immunotherapy, n (%)	13 (32.5%)
Concurrent hematological malignancy	8 (20%)
Concurrent hematological malignancy/Prior Chemo-/radio-/immuno-therapy	17 (42.5%)
Gender corrected VAF %, Median (Q1, Q3)	31.5 (20.5, 40.8)
Isolated *ZRSR2*, n (%)	6 (15%)
Multiple *ZRSR2*, n (%)	1 (2.5%)
Number of co-mutations, Median (Q1, Q3)	1 (1, 1.5)
TET2m, n (%)	25 (62.5%)
Progression to myeloid neoplasms, n (%)	9 (22.5%)
Progression to MDS	5 (12.5%)
CCUS risk (Per CHRS)	
Intermediate risk, n (%)	3 (7.5%)
High risk, n (%)	37 (92.5%)
Progression to MDS (p=0.005)	
Treated CCUS	1 (3.5%)
Untreated CCUS	4 (36.4%)

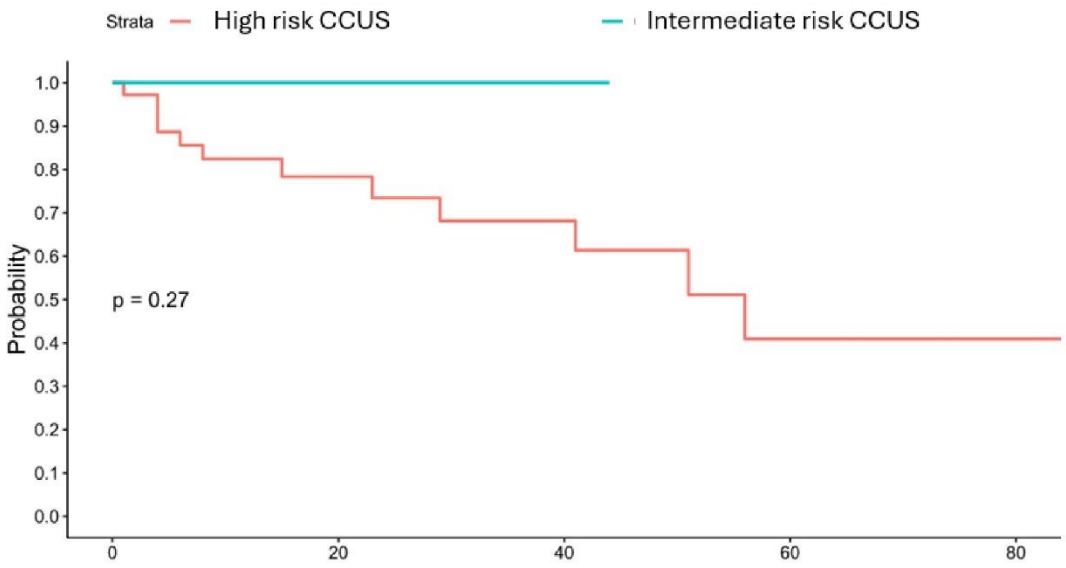
Legend

Figure 1. (A) Overall survival for 40 *ZRSR2m* CCUS patients (B) Overall survival of *ZRSR2m* CCUS patients with isolated *TET2* co-mutations vs *ZRSR2m* CCUS patients with other co-mutations (C) Bar chart showing number of patients progressing to MDS among treated and untreated *ZRSR2m* CCUS patients


A
Overall survival
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.0
0
20
40
60
80
Time (months)
B
Others
Isolated TET2
Overall survival
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
P= 0.025
0
20
40
60
80
Time (months)
C
35
30
25
20
15
10
5
0
No treatment
Treatment
Did not progress to MDS
Progressed to MDS



Supplementary Figure 1. Distribution of co-mutations in *ZRSR2*-mutated CCUS patients.



Supplementary Figure 2. Overall survival stratified by clonal hematopoiesis risk score in *ZRSR2*-mutated CCUS.