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MYELODYSPLASTIC NEOPLASM

Characterisation and prognostic impact Of *ZRSR2* mutations in myeloid neoplasms

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TO THE EDITOR:

The *ZRSR2* gene, located on the X chromosome (Xp22.1), is a member of the RNA splicing machinery family of genes which also includes *SF3B1*, *SRSF2*, and *U2AF1* [1–3]. Spliceosome gene mutations are found in 35% of myelodysplastic syndrome (MDS) patients and were described as being mutually exclusive with each other [3, 4]. *ZRSR2m* is seen in 4% of MDS, and across different myeloid neoplasms (MN) including acute myeloid leukemia (AML), chronic myelomonocytic leukemia (CMML), myeloproliferative neoplasm (MPN) [1, 5–7]. Damm et al. found that mutations in *ZRSR2* were evenly distributed across the entire gene, and included nonsense, frameshift, and splice site mutations [1, 2]. The only co-mutation that showed significant association with *ZRSR2* was *TET2* ($p < 0.001$) [2, 8, 9]. *ZRSR2m* cells showed increased precursor cells for macrophages and decreased precursor cells for erythroid cells [10]. Malcovati et al. reported that *TET2*, *ZRSR2* co-mutation was predictive of myelomonocytic phenotype and showed higher hemoglobin (Hgb) levels and monocyte counts [11].

This is a retrospective study done with IRB approval at our institution. No interaction was done with patients. A waiver of consent was approved by our IRB due to minimal risk nature. No cases of age younger than 18 were included. This study was conducted in a single institution. All methods carried out in our study were in accordance with relevant guidelines and regulations. Next-generation sequencing (NGS) was performed in the molecular hematopathology laboratory with the NGS gene panel including 42-47 genes, between 2016-2023. BlueSky Statistics V10.3.1 was used for data analysis.

NGS was performed clinically on 9320 samples, 164 had the *ZRSR2m* genotype, with only 2 being female (1.2%). Median patient age was 74 (range 31–92). The most common diagnosis was MDS (n = 53, 32.3%), clonal cytopenia of undetermined significance (CCUS) (n = 39, 23.8%), MPN (n = 33, 20.1%), MDS/MPN overlap (n = 23, 14%), AML (n = 15, 9.1%) and 1 mixed phenotype acute leukemia (MPAL). Fifteen (9%) patients had concurrent non-myeloid hematological malignancies diagnosed at the time of the NGS. Only 15 patients (9.1%) received prior chemotherapy or radiotherapy, 29 patients (17.7%) received prior immunotherapy with the highest frequency among CCUS patients (n = 13, 33%) (Supplementary Table 1). Abnormal cytogenetics were found in 54 patients (33%), with +8 (16) and -Y (11) being the most common (Supplementary Table 2).

Seventy-eight patients (48%) were diagnosed before our in-house NGS (Supplementary Table 3). Out of 10 patients diagnosed with CCUS, 8 (80%) progressed to MDS by the time of the NGS, and two (7.7%) and 5 (11.5%) MDS patients progressed to AML and MDS/MPN overlap, respectively.

The most common subtype among MDS was low blast (MDS-LB) (n = 37, 69.8%). Eighteen of 23 MDS/MPN Overlap were CMML (78%) and the most common MPN subtype was myelofibrosis (MF) (n = 27, 81.8%).

The most common risk stratification among MDS patients by IPSS-M scoring was low risk (n = 16, 30.2%) (Supplementary Figs. 1, 2, 3, 4). Twenty-four (45.3%) of MDS patients were stratified as low risk according to IPSS-R (Supplementary Table 4). Twenty-seven MDS patients (51%), and 23 CCUS patients (59%) had absolute monocyte count $\geq 0.5 \times 10^9$/L (Supplementary Table 1).

Median Gender-corrected VAF was 35 (range, 1–66). and multiple *ZRSR2* mutations were found in 7 patients (4.3%).

Mutations occurred in Pre-ZF1 (49%), UHM (27%), and Post-ZF2 (13%) domains and were spread across the entire length of the gene. (Fig. 1A, Supplementary Fig. 5A, Supplementary Table 5, Supplementary Table 6) The most common mutation type was nonsense (n = 69, 42%) (Supplementary Fig. 5B, Supplementary Table 5).

The median number of co-mutations was 2 (range, 0–6). A significant correlation was found between MN classification and number of co-mutations in *ZRSR2m* patients ($p < 0.001$) (Supplementary Fig. 7).

The most common co-mutation was *TET2* which was present in 84 patients (51%) (42% had multiple *TET2*) (Fig. 1B, Supplementary Figs. 8, 9, Supplementary Table 7) A significant correlation was found between MN classification and presence of *TET2* in *ZRSR2m*

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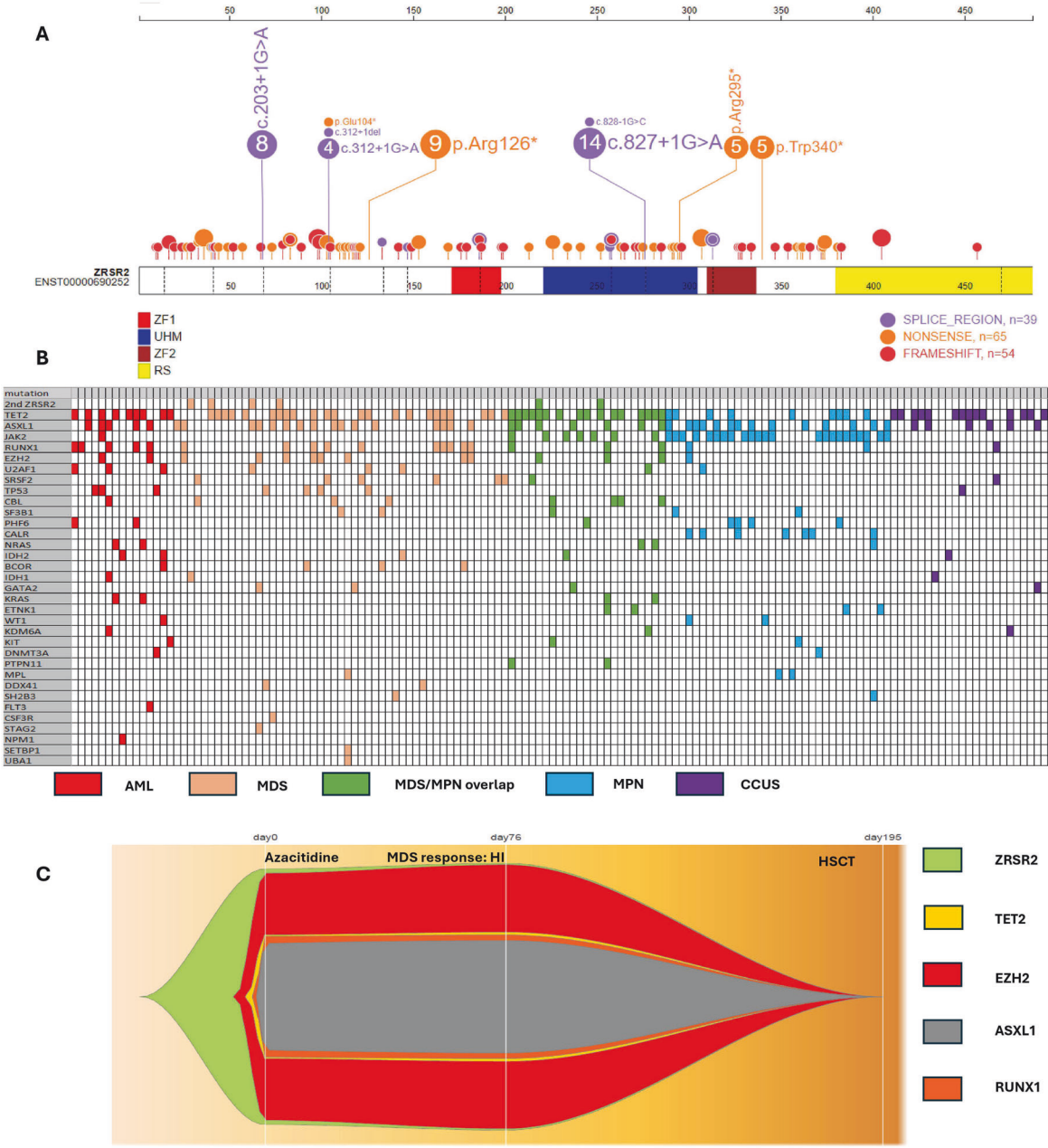

Fig. 1 *ZRSR2* mutations, co-mutations and clonal dynamics with treatment. A Representation of *ZRSR2* mutations detected, positioned on the ZRSR2 protein and its functional domains. **B** The co-mutational pattern in 164 *ZRSR2m* MN patients. A column represents each patient. **C** Fishplot showing the progression of mutations VAF in *ZRSR2m* MDS patients throughout disease course and therapy.

patients (p = 0.007) (highest frequency among MDS/MPN overlap, 70%). Other common co-mutations were *ASXL1* (n = 52, 32%), and *JAK2* (n = 31, 19%) (Supplementary Fig. 8, Supplementary Table 8). A significant correlation was also found between MN classification and *RUNX1* co-mutations (p = 0.008), and they were found with the highest prevalence among AML (n = 5, 33%) and MDS (n = 12, 23%).

Other members of the spliceosome family of genes were present in 14.7% of patients, including *U2AF1* (n = 9, 5.5%), *SRSF2* (n = 8, 5%), and *SF3B1* (n = 7, 4%).

Only 13 patients (4.3%) had isolated *ZRSR2* mutations. Median IPSS-M score was lower in patients with isolated *ZRSR2m* (−1.18) compared to patients with co-mutations (–0.74) (p = 0.02).

108 patients (66%) received treatment. 8 AML patients (66.7%) achieved response to therapy with 2 (16.7%) complete remissions with incomplete hematologic recovery (CRi), and 6 (50%) with CR, of whom 1 relapsed (Supplementary Fig. 10: B). Among 39 *ZRSR2m* MDS receiving treatment, 7 (18%) had hematological improvement (HI), 3 (7.7%) had complete remission with limited recovery (CR_L), 1 CR equivalent (2.6), and 3 (7.7%) had CR, of which 2 relapsed (Supplementary Fig. 10: A). Hematopoietic stem cell transplant (HSCT) was performed in 21 patients (12.8%).

The most used medications were hypomethylating agents (HMA) (Supplementary Fig. 11, Supplementary Table 9). Fig. 1C shows the progression of mVAF in a *ZRSR2m* MDS patient among ZRSR2 and the co-mutations and their changes after therapy and HSCT.

Thirteen patients (8.7%) progressed to AML, 9 from MDS (17%, reported average is 30-40%) 2 from MDS/MPN overlap (8.7%), and 2 from MPN (6%), none of these patients had isolated

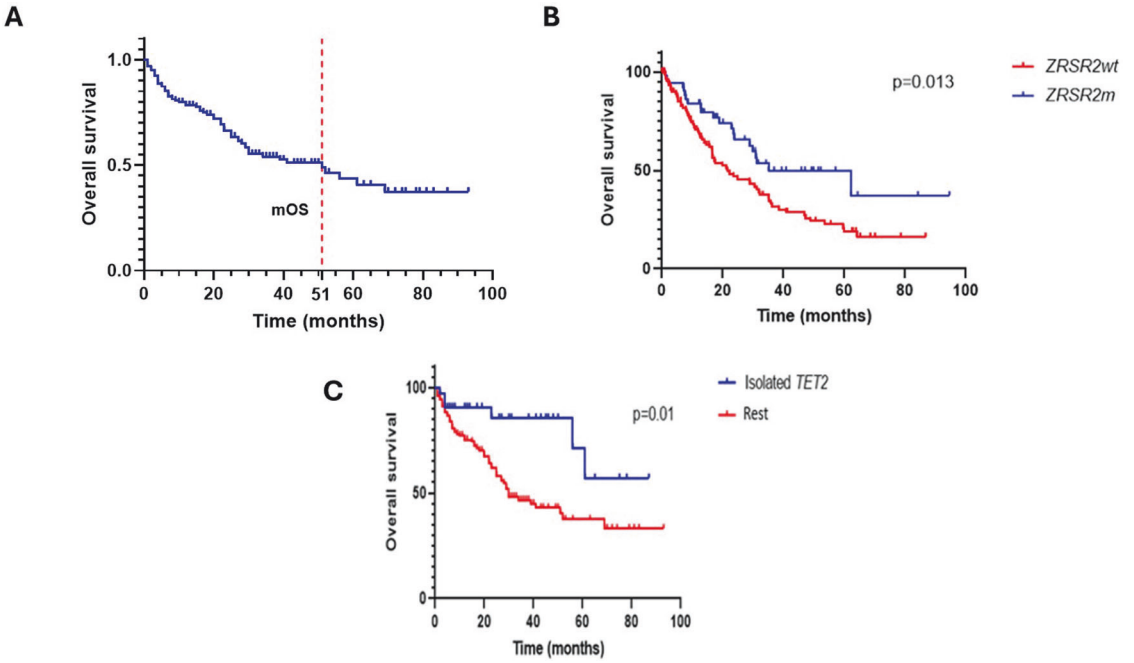

Fig. 2 Overall survival of patients with myeloid neoplasm based on presence or lack of *ZRSR2* mutation and other co-mutations. A Overall survival for 164 *ZRSR2m* MN patients (with dotted red line showing median OS). **B** OS of *ZRSR2m* vs *ZRSRwt* in MDS patients. **C** OS according to the presence of an isolated *TET2* co-mutation in *ZRSR2m* MN patients.

ZRSR2 and only 1 MDS patient had isolated *TET2* as a co-mutation. [25,26] Ten patients (8%) progressed into CMML or MDS/MPN overlap from CCUS, MDS, or MPN.

There were 68 deaths, with median overall survival (mOS) 51 months, and median follow-up of 35 months. (Fig 2A) *ZRSR2m* MDS patients had better mOS compared to the MDS control group with *ZRSR2wt* (35 months vs 22 months, $p = 0.013$). (Fig 2B) mOS of *ZRSR2m* patients varied significantly among different MNs ($p = 0.004$) (Supplementary Fig. 12).

ZRSR2m with spliceosome and tumor suppressor gene (TSG) co-mutations showed worse survival (25 vs 56 months, $p = 0.02$ and 20 vs 51 months, respectively, $p = 0.04$) (Supplementary Figs. 10, 13). Patients with *TET2* as an isolated co-mutation had better survival (not reached vs 30 months, $p = 0.01$). (Fig. 2C) Patients with *RUNX1* co-mutations had worse survival (28 vs 52 months, $p = 0.02$) (Supplementary Fig. 13).

Improved survival was seen in patients with PB blasts <5% (52 months vs 9 months, HR = 0.027, $p < 0.001$) and higher Hgb concentration (HR = 0.78, $p < 0.001$), while patients with increased WBCs count (HR = 1.02, $p < 0.001$), absolute neutrophil count (ANC) (HR = 1.04, $p = 0.01$), absolute monocyte count (AMC) (HR = 1.2, $p = 0.008$), BM blasts (HR = 1.02, $p < 0.001$), PB blasts (HR = 1.03, $p < 0.001$) showed worse mOS (Supplementary Fig. 14). Patients with higher number of co-mutations (HR = 1.49, $p < 0.001$) and patients with abnormal cytogenetics (25 vs 61 months, $p < 0.001$) showed worse OS (Supplementary Fig. 15). On multivariate analysis, only higher Hgb concentration (HR = 0.8, $p = 0.004$), PB blasts>5% (HR = 2.2, $p = 0.02$), and abnormal cytogenetics (HR = 1.9, $p = 0.01$) retained significance (Supplementary Table 10).

Sequential NGS (S1-NGS) was performed in 55 out of the 164 patients (33.5%), 50 (91%) of them continued to have *ZRSR2m*. Out of the 21 patients who had HSCT, 4 (19%) performed sequential NGS post-transplant and all of them had negative NGS. mVAF for *ZRSR2m* was 80% and 89% for S1-NGS and S2-NGS, respectively, showing a statistically significant increase of 11% for S1-NGS from the first NGS ($p = 0.01$).

Our study's cohort had only 2 female patients, data by Daichi Inoue et al. suggest that *ZRSR2* escapes from X inactivation and is not pathogenic in females at heterozygous state [2, 12]. We also find that MDS patients with *ZRSR2* mutations have better survival, indicating a favorable prognosis.

Our study found a notable association between *ZRSR2m* and a higher incidence of CCUS diagnoses, which is a novel finding. Our findings suggest that the *ZRSR2m* mutation carries a favorable prognosis among MNs, especially in isolated *TET2m* group.

Our findings clinically support Madan et al. findings as we found that over half of the *ZRSR2m* MDS and CCUS cohort had elevated absolute monocyte count ($\geq 0.5 \times 10^9$/L), majority of MDS/MPN overlap patients were diagnosed as CMML, and about 8% of patients originally diagnosed as CCUS, MDS or MPN progressed or were re-diagnosed as CMML later [10].

Interestingly, MPN was found in 20% of *ZRSR2m* patients and consisted mainly of MF, which raises the possibility of acquiring *ZRSR2m* later in MPN progression into MF. Our study demonstrated a strong association between *ZRSR2m* and *TET2m* (51% of patients) and that the presence of *TET2*m as an isolated co-mutation was shown to be associated with longer survival and a higher prevalence among MDS/MPN overlap patients. Other studies reported enrichment of *ZRSR2* in spliceosome mutated cases and our paper supports this (10.4% of our *ZRSR2m* MN patients had either *U2AF1* (5.5%) or *SRSF2* (4.9%)) [3, 13].

Survival among *ZRSR2m* MN patients was affected mostly by the MN diagnosis (AML showing worst survival) and expectedly by PB blasts >5%. Other factors that affected survival positively were higher Hgb concentration and the presence of isolated *TET2m*. On the contrary, presence of *RUNX1m* and cytogenetic abnormalities affected survival negatively.

Our study was limited by data collection from a single institution, the retrospective nature, shorter follow-up duration, small cohort size (due to gene rarity), and delayed NGS introduction in some cases. Furthermore, our focus was on clinical aspects of this mutation and lacked mechanistic insights into this disease.

In conclusion, the *ZRSR2m* was almost exclusively seen in males, with a striking increased frequency of CCUS patients. *TET2m* was the most common co-mutation and is linked to better survival especially as an isolated co-mutation. Over half of the patients with *ZRSR2m* MDS and CCUS had a higher absolute monocyte count indicating a possible association with monocytic differentiation. However, further studies are needed to confirm these findings.

DATA AVAILABILITY

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

REFERENCES

1. Yoshida K, Sanada M, Shiraishi Y, Nowak D, Nagata Y, Yamamoto R, et al. Frequent pathway mutations of splicing machinery in myelodysplasia. Nature. 2011;478:64–9.
2. Damm F, Kosmider O, Gelsi-Boyer V, Renneville A, Carbuccia N, Hidalgo-Curtis C, et al. Mutations affecting mRNA splicing define distinct clinical phenotypes and correlate with patient outcome in myelodysplastic syndromes. Blood. 2012;119:3211–8.
3. Thol F, Kade S, Schlarmann C, Löffeld P, Morgan M, Krauter J, et al. Frequency and prognostic impact of mutations in SRSF2, U2AF1, and ZRSR2 in patients with myelodysplastic syndromes. Blood. 2012;119:3578–84.
4. Chiereghin C, Travaglino E, Zampini M, Saba E, Saitta C, Riva E, et al. The genetics of myelodysplastic syndromes: clinical relevance. Genes (Basel). 2021;12:1144.
5. AACR Project GENIE: powering precision medicine through an international consortium. Cancer Discov, 2017;7:818–31.
6. Hosono N. Genetic abnormalities and pathophysiology of MDS. Int J Clin Oncol. 2019;24:885–92.
7. Togami K, Chung SS, Madan V, Booth CAG, Kenyon CM, Cabal-Hierro L, et al. Sex-biased ZRSR2 mutations in myeloid malignancies impair plasmacytoid dendritic cell activation and apoptosis. Cancer Discov. 2022;12:522–41.
8. Papaemmanuil E, Gerstung M, Malcovati L, Tauro S, Gundem G, Van Loo P, et al. Clinical and biological implications of driver mutations in myelodysplastic syndromes. Blood. 2013;122:3616–27.
9. Garcia-Ruiz C, Martínez-Valiente C, Cordón L, Liquori A, Fernández-González R, Pericuesta E, et al. Concurrent Zrsr2 mutation and Tet2 loss promote myelodysplastic neoplasm in mice. Leukemia. 2022;36:2509–18.
10. Madan V, Kanojia D, Li J, Okamoto R, Sato-Otsubo A, Kohlmann A, et al. Aberrant splicing of U12-type introns is the hallmark of ZRSR2 mutant myelodysplastic syndrome. Nat Commun. 2015;6:6042.
11. Malcovati L, Papaemmanuil E, Ambaglio I, Elena C, Gallì A, Della Porta MG, et al. Driver somatic mutations identify distinct disease entities within myeloid neoplasms with myelodysplasia. Blood. 2014;124:1513–21.
12. Inoue D, Polaski JT, Taylor J, Castel P, Chen S, Kobayashi S, et al. Minor intron retention drives clonal hematopoietic disorders and diverse cancer predisposition. Nat Genet. 2021;53:707–18.
13. Taylor J, Mi X, North K, Binder M, Penson A, Lasho T, et al. Single-cell genomics reveals the genetic and molecular bases for escape from mutational epistasis in myeloid neoplasms. Blood. 2020;136:1477–86.

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The protein diagram was generated using ProteinPaint (https://proteinpaint.stjude.org/). The Fishplots depicting clonal evolution were generated using https://github.com/chrisamiller/fishplot.

AUTHOR CONTRIBUTIONS

MY, BK, YJ, and AA planned the study, reviewed data, performed statistical analysis, and wrote the manuscript. RH and DV performed molecular analysis and reviewed the manuscript. PG performed cytogenetic analysis and reviewed the analysis. KB coordinated NGS data collection. DJ, JF, JP, AS, MH, KB, WH, MP, MS, and HA reviewed the paper and contributed patients.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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